(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) **EP 2 011 495 A1**

(12) **EUROPEAN PATENT APPLICATION**

(43) Date of publication:
07.01.2009 Bulletin 2009/02

(21) Application number: **07012964.8**

(22) Date of filing: **03.07.2007**

(51) Int Cl.:
A61K 31/343 *(2006.01)* ***A61K 31/4015*** *(2006.01)*
A61K 31/4025 *(2006.01)* ***A61K 31/45*** *(2006.01)*
A61K 31/4525 *(2006.01)* ***A61K 31/5375*** *(2006.01)*
A61K 31/5377 *(2006.01)* ***A61K 36/67*** *(2006.01)*
A61P 25/00 *(2006.01)* ***A61P 25/02*** *(2006.01)*
A61P 25/18 *(2006.01)* ***A61P 25/28*** *(2006.01)*
A61P 27/06 *(2006.01)*

(84) Designated Contracting States:
AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IS IT LI LT LU LV MC MT NL PL PT RO SE SI SK TR
Designated Extension States:
AL BA HR MK RS

(71) Applicant: **Sygnis Bioscience GmbH & Co. KG**
69120 Heidelberg (DE)

(72) Inventors:
- **Schneider, Armin**
  69118 Heidelberg (DE)
- **Moraru, Anja**
  68199 Mannheim (DE)
- **Krüger, Carola**
  67434 Neustadt/Wstr. (DE)
- **Laage, Rico**
  69198 Schriesheim (DE)
- **Pitzer, Claudia**
  69231 Rauenberg (DE)

(74) Representative: **Dick, Alexander et al**
Isenbruck Bösl Hörschler Wichmann Huhn Patentanwälte
Im Neuenheimer Feld 582
69120 Heidelberg (DE)

(54) **Use of piperine and derivatives thereof for the therapy of neurological conditions**

(57) The present invention relates to the use of piperine and derivatives thereof for the preparation of a pharmaceutical composition for treating and/or preventing a neuronal condition where there is a need of neuroprotection and/or neuroregeneration. The invention furthermore relates to the use of piperine and derivatives thereof for the in vitro differentiation of neuronal stem cells and the use of such pre-treated cells for stem cell therapy von neurological conditions.

EP 2 011 495 A1

Description

[0001] The present invention relates to the use of piperine and derivatives thereof for the manufacture of a medicament for the treatment and/or prophylaxis of neurological and/or psychiatric conditions. The invention furthermore relates to the use of piperine and derivatives thereof for the in vitro differentiation of neuronal stem cells and the use of such pre-treated cells for stem cell therapy von neurological conditions.

[0002] Neurological conditions are medical conditions including diseases or disorder which affects the nervous system. The neurological condition may be accompanied by an pathologically impaired function of the nervous system, e.g., due to a disease or disorder, or may simply require an improvement of the neurological functions as achieved, e.g., by enhancing the cognitive ability in order to improve learning and memory.

[0003] Examples of such neurological conditions are diseases with a patho-physiological mechanisms of cerebral ischemia or hypoxia include stroke (as well as hemorrhagic stroke), cerebral microanglopathy (small vessel disease), intrapartal cerebral, ischemia, cerebral ischemia during/after cardiac arrest or resuscitation, cerebral ischemia due to intraoperative problems, cerebral Ischemia during carotid surgery, chronic cerebral ischemia due to stenosis of blood-supplying arteries to the brain, sinus thrombosis or thrombosis of cerebral veins, cerebral vessel malformations, and diabetic retinopathy. Further examples of these neurological conditions include amyotrophic lateral sclerosis (ALS), Huntington's disease, Wilson's disease, multi-system atrophy, Alzheimer's disease, Glaucoma, Pick's disease, Lewy-body disease, Hallervorden-Spatz disease, torsion dystonia, hereditary sensorimotor neuropathies (HMSN), Gerstmann-Strussier-Schanker disease, Creutzfeld-Jakob-disease, Machado-Joseph disease, Friedreich ataxia, Non-Friedreich ataxias, Gilles de la Tourette syndrome, familial tremors, olivopontocerebellar degenerations, paraneoplastic cerebral syndromes, hereditary spastic paraplegias, hereditary optic neuropathy (Leber), retinitis pigmentosa, Stargardt disease, and Kearns-Sayre syndrome and septic shock, intracerebral bleeding, subarachnoidal hemorrhage, multlinfarct dementia, Inflammatory diseases (such as vasculitis, multiple sclerosis, and Guillain-Barre-syndrome), neurotrauma (such as spinal cord trauma, and brain trauma), peripheral neuropathies, polyneuropathies, schizophrenia, depression, metabolic encephalopathies, and Infections of the central nervous system (viral, bacterial, fungal).

[0004] Most studies on cerebral ischemia and testing of pharmacological substances in vivo have only been concerned with the immediate effects of the drug or paradigm under investigation (i.e. infarct size 24 h after induction of the stroke). However, a more valid parameter of true efficacy of a particular substance is the long-term effect on functional recovery, which is also reflected in human stroke studies, where clinical scales (e.g., Scandinavian stroke scale, NIH scale, Barthel index) also reflect the ability to perform daily life activities. Recovery in the first few days after focal lesions may be due to resolution of edema or reperfusion of the ischemic penumbra. Much of the functional recovery after the acute phase is likely due to brain plasticity, with adjacent cortical areas of the brain taking over functions previously performed by the damaged regions *(*Chen et al., Neuroscience 2002; 111: 761-773). The two main mechanisms proposed to explain reorganization are unmasking of previously present but functionally inactive connections and growth of new connections such as collateral sprouting *(*Chen et al., Neuroscience 2002; 111: 761-773). Short term plastic changes are mediated by removing inhibition to excitatory synapses, which Is likely due to reduced GABAergic inhibition *(*Kaas, Annu Rev Neurosci. 1991; 14: 137-167*;* Jones, Cereb Cortex. 1993; 3: 361-372*).* Plasticity changes that occur over a longer time involve mechanisms in addition to the unmasking of latent synapses such as long-term potentiation (LTP), which requires NMDA receptor activation and increased intracellular calcium concentration (Hess & Donoghue, Neurophysiol. 1994; 71: 2543-2547*),* Long term changes also involve axonal regeneration and sprouting with alterations in synapse shape, number, size and type *(*Kaas, Annu Rev Neurosci. 1991; 14: 137-167*).* Recent Investigations show that the enhancement of neuroregenerative processes after cerebral ischemia improve the outcome *(*Fisher et al. Stroke 2006; 37: 1129-1136*).* There is a compelling need to develop cell and pharmacological therapeutic approaches to be administered beyond the hyperacute phase of stroke. Therefore, a successful future stroke therapy which is designed to reduce neurological deficits after stroke should approach besides revascularization at once also prevention of cell death, stimulation of neuroregeneration, and plasticity.

[0005] Cerebral ischemia may result from a variety of causes that impair cerebral blood flow (CBF) and lead to deprivation of both oxygen and glucose. Traumatic brain injury (TBI), on the other hand, involves a primary mechanical impact that usually causes skull fracture and abruptly disrupts the brain parenchyma with shearing and tearing of blood vessels and brain tissue. This, in turn, triggers a cascade of events characterized by activation of molecular and cellular responses that lead to secondary Injury. The evolution of such secondary damage is an active process in which many biochemical pathways are involved *(*Leker & Shohami, Brain Res. Rev. 2002; 39: 55-73*).* Many similarities between the harmful pathways that lead to secondary cellular death in the penumbral ischemic zone and in the area exposed to secondary post-traumatic Injury have been identified (e.g. excitotoxity by excess glutamate release, nitric oxide, reactive oxygen species, inflammation, and apoptosis (Leker & Shohami, Brain Res. Rev. 2002; 39: 55-73)). In addition, early ischemic episodes are reported to occur after traumatic brain injury, adding a component of ischemia to the primary mechanical damage.

[0006] Stroke is the third-leading cause of death, and the main cause of disability in the western world. It presents a

large socio-economic burden. The etiology can be either ischemic (in the majority ur cases) or hemorraghic. The cause of ischemic stroke is often embolic, or thrombotic. So far, there is no effective treatment for the majority of stroke patients. The only clinically proven drugs so far are tissue plasminogen activator (TPA) and Aspirin. After massive cell death in the immediate infarct core due to lack of glucose and oxygen, the infarct area expands for days, owing to secondary mechanisms such as glutamate excitotoxicity, apoptotic mechanisms, and generation of free radicals.

[0007] Cardiovascular disease is the major cause of death in western industrialized nations. In the United States, there are approximately 1 million deaths each year with nearly 50% of them being sudden and occurring outside the hospital *(*Zheng et al., Circulation 2001; 104: 2158-2163*).* Cardiopulmonary resuscitation (CPR) is attempted in 40-90 of 100,000 inhabitants annually, and restoration of spontaneous circulation (ROSC) is achieved in 25-50% of these patients. However, the hospital discharge rate following successful ROSC is only 2-10% *(*Bottiger et al., Heart 1999; 82: 674-679). Therefore, the vast majority of the cardiac arrest victims annually in the United states Is nuL treated successfully. The major reason fnr the low survival rates after successful CPR, i.e., for post-arrest in-hospital mortality, is persistent brain damage. Brain damage following cardiocirculatory arrest is related both to the short period of tolerance to hypoxic stress and to specific reperfusion disorders *(*Safar, Circulation 1986; 74: UV138-153*,* Hossmann, Resuscitation 1993; 26: 225-235). Initially, a higher number of patients can be stabilized hemodynamically after cardiocirculatory arrest; many of them, however, die due to central nervous system injury. The personal, social, and economic consequences of brain damage following cardiac arrest are devastating. One of the most Important issues in cardiac arrest and resuscitation ("whole body ischemia and reperfusion") research, therefore, is cerebral resuscitation and post-arrest cerebral damage *(*Safar, Circulation 1986; 74: UV138-153*,* Safar et al., Crit Care Med 2002; 30: 140-144*).* Presently, it is not possible to decrease the primary damage to neurons that is caused by hypoxia during cardiac arrest by any post-arrest therapeutic measures. Major patho-physiological issues include hypoxia and subsequent necrosis, reperfusion injury with free radical formation and cellular calcium influx, release of excitatory amino acids, cerebral microcirculatory reperfusion disorders, and programmed neuronal death or apoptosis *(*Safar, Circulation 1986; 74: UV138-153*,* Safar et al., Crit Care Med 2002; 30: 140-144*).*

[0008] Amyotrophic lateral sclerosis (ALS; Lou-Gehrig's disease; Charcot's disease) is a neurodegenerative disorder with an annual incidence of 0.4 to 1.76 per 100.000 population *(*Adams et al., Principles of Neurology, 6.sup.th ed., New York, pp 1090 1095*).* It is the most Common form of motor neuron disease with typical manifestations of generalized fasciculations, progressive atrophy and weakness of the skeletal muscles, spasticity and pyramidal tract signs, dysarthria, dysphagia, and dyspnea. The pathology consists principally in loss of nerve cells in the anterior horn of the spinal cord and motor nuclei of the lower brainstem, but can also include the first order motor neurons in the cortex. Pathogenesis of this devastating disease is still largely unknown, although the role of superoxide-dismutase, (SOD1) mutants in familial cases has been worked out quite well, which invokes an oxidative stress hypothesis. So far, more than 90 mutations in the SOD1 protein have been described, that can cause ALS *(*Cleveland & Rothstein, Nat Rev Neurosci. 2001; 2: 806-819*).* Also, a role for neurofilaments in this disease was shown. Excitotoxicity, a mechanism evoked by an excess glutamate stimulation is also an important factor, exemplified by the beneficial role of Riluzole in human patients. Most convincingly shown in the SOD1 mutants, activation of caspases and apoptosis seems to be the common final pathway in ALS *(*Ishigaki et al., J Neurochem. 2002; 82: 576 584*.,* Li et al., Science 2000; 288 : 335-339). Therefore, it seems that ALS also falls into the same general pathogenetic pattern that is also operative in other neurodegenerative diseases and stroke, i.c. glutamate involvement, oxidative stress, and programmed cell death.

[0009] Glaucoma is the number one cause of preventable blindness in the United States. Glaucoma is a group of conditions where the nerve of sight (the optic nerve) is damaged, usually as a result of increased pressure within the eye, but glaucoma can also occur with normal or even below-normal eye pressure. The lamina cribrosa (LC) region of the optic nerve head (ONH) is a major site of injury in glaucomatous optic neuropathy. It is a patchy loss of vision, which is permanent, but progress of the condition can be minimised if it is detected early enough and treatment is begun. However, if left untreated, glaucoma can eventually lead to blindness. Glaucoma is one of the most common eye disorders amongst older people. Worldwide, it is estimated that about 66.8 million people have visual impairment from glaucoma, with 6.7 million suffering from blindness. There are a variety of different types or glaucoma. The most common forms are: Primary Open-Angle Glaucoma; Normal Tension Glaucoma; Angle-Closure Glaucoma; Acute Glaucoma; Pigmentary Glaucoma; Exfoliation Syndrome or Trauma-Related Glaucoma. Glaucoma can be treated with eyedrops, pills, laser surgery, eye operations, or a combination of methods. The whole purpose of treatment is to prevent further loss of vision. This is imperative as loss of vision due to glaucoma is irreversible. Keeping the IOP under control is the key to preventing loss of vision from glaucoma. Recent developments emphasises the requirement of neuroprotection and neuroregeneration for the treatment of glaucoma. *(*Levin, Ophthalmol Clin North Am. 2005;18:585-596, vii; Schwartz et al., J Glaucoma. 1996; 5: 427-432*).*

[0010] Alzheimer's disease (AD) is a neurodegenerative disease characterized by progressive cognitive deterioration together with declining activities of daily living and neuropsychiatric symptoms or behavioral changes. It is the most common type of dementia. The most striking early symptom is loss of memory, which usually manifests as minor forgetfulness that becomes steadily more pronounced with illness progression, with relative preservation of older mem-

ories. The pathological process consists principally of neuronal loss or atrophy, principally in the temporoparietal cortex, but also in the frontal cortex, together with an inflammatory response to the deposition of amyloid plaques and neurofibrillary tangles. The ultimate cause of the disease is unknown. Three major competing hypotheses exist to explain the cause of the disease. The oldest, on which most currently available drug therapies are based, is known as the "cholinergic hypothesis" and suggests that AD is due to reduced biosynthesis of the neurotransmitter acetylcholine. Levels of acetylcholine are reduced in brain tissue or AD patients, whereas glutamate levels are usually elevated. The medications that treat acetylcholine deficiency have served to only treat symptoms of the disease and have neither halted nor reversed it *(*Walker & Rosen, Age Ageing 2006; 35:332-335). Research after 2000 includes hypotheses centred on the effects of the misfolded and aggregated proteins, amyloid beta and tau. The two positions differ with one stating that the tau protein abnormalities initiate the disease cascade, while the other believes that beta amyloid deposits are the causative factor in the disease (Mudher & Lovestone, Trends Neurosci. 2002; 25: 22-26).

[0011] There is currently no cure for AD. Currently available medications offer relatively small symptomatic benefit for some patients but do not slow disease progression. It helps a little for the memory *(*Lyketsos et al., Am J Geriatr Psychiatry. 2006; 14:561-572). Acetylcholinesterase (AChE) inhibition was thought to be important because there is a reduction in activity of the cholinergic neurons. Acetylcholinesterase-inhibitors seemed to modestly moderate symptoms but do not alter the course of the underlying dementing process. There is significant doubt as to the effectiveness of cholinesterase inhibitors. One of the natural extracts that has been examined in AD is Ginkgo *(Ginkgo biloba*). A large, randomized clinical study in the US is underway and examining the effect of Ginkgo to prevent dementia (DeKosky et al., Contemp Clin Trials 2006, 27: 238-253*).* Recent evidence of the involvement of glutamatergic neuronal excitotoxicity causes AD led to the development and introduction of memantine, a novel NMDA receptor antagonist, which has been shown to be moderately clinically efficacious *(*Areosa-Sastre et al., Cochrane Database Syst Rev. 2004; 18: CD003154*).* Recent investigations on the processes of neurogenesis and the observation of widespread existence of endogenous neural progenitors offers hope that the potential of these cells may be harnessed to repair neurodegenerative diseases such as AD *(*Flder et al., Mt Sinai J Med. 2006; 73:931-940*;* Brinton & Wang, Curr Alzheimer Res. 2006; 3:185-190*;* Kelleher-Andersson, Curr Alzheimer Res. 2006; 3: 55-62*;* Greenberg & Jin, Curr Alzheimer Res. 2006; 3: 25-8*).*

[0012] One group of Neurodegenerative disorders is characterized by an expansion of trinucleotides. Those neurodegenerative trinucleotide repeat disorders are chronic and progressive characterised by selective and symmetric loss of neurons in motor, sensory, or cognitive systems. Symptoms are often ataxia, dementia or motor dysfunction. The best known trinucleotide repeat disorder is Huntington's disease, others are Spinal and bulbar muscular atrophy (Kennedy's disease), Autosomal dominant spinocerebellar ataxia's: Type 1 SCA1, Type 2 SCA2, Type 3 (Machado-Joseph disease) SCA3/MJD, Type 6 SCA6, Type 7 SCA7, Type 8 SCA8, Friedreich's Ataxia and Dentatorubral pallidoluysian atrophy DRPLA/Haw-River syndrome. *(*Hardy & Gwinn-Hardy, Science 1998; 282: 1075-1079*;* Martin, N Engl J Med, 1999; 340: 1970-1980*;* Schols et al., Ann Neurol. 1997; 42: 924-932*).*

[0013] Huntington's discase (HD) is an autosomal dominant, inherited, neuropsychiatric disease which gives rise to progressive motor, cognitive and behavioural symptoms. The course of HD is Characterized by jerking uncontrollable movement of the limbs, trunk, and face (chorea); progressive loss of mental abilities; and the development of psychiatric problems. HD progresses without remission over 10 to 25 years and usually appears in middle age (30-50 years). Juvenile HD (also called Westphal variant or akinetic-rigid HD) develops before the age of 20, progresses rapidly, and produces muscle rigidity in which the patient moves little, if at all (akinesia). It is estimated that one in every 10,000 persons - nearly 30,000 in the United States - have HD. Juvenile HD occurs in approximately 16% of all cases. Its core pathology involves degeneration of the basal ganglia, in particular, the caudate and putamen, and is caused by an unstable expansion of the trinucleotide CAG, coding for glutamine, in a single autosomal gene IT-15 on chromosome 4, coding for a mutated form of the protein, Huntingtin. How the mutation of gene IT-15 alters the function of the protein is not well understood.

[0014] Treatment of HD focuses on reducing symptoms, preventing complications, and providing support and assistance to the patient. There are several substances available today for the treatment of chorea. Other neurological symptoms, such as dystonia, can be treated, but treatment is associated with a high risk of adverse events. Psychiatric symptoms, on the other hand, are often amenable to treatment and relief of these symptoms may provide significant improvement in quality of life. *(*Bonelli & Hofmann, Expert Opin Pharmacother. 2004; 5: 767-776*). Most* drugs used to treat the symptoms of HD have side effects such as fatigue, restlessness, or hyperexcitability. Cystamine (=Decarboxycystine) alleviates tremors and prolongs life in mice with the gene mutation for HD. The drug appears to work by increasing the activity of proteins that protect nerve cells, or neurons, from degeneration. The study suggests that a similar treatment or a neuroregenerative treatment may one day be useful in humans with HD and related disorders. *(*Karpuj et al., Nat Med. 2002; 8: 143 149*).*

[0015] The lysosomal storage diseases (LSD) are a group of about 40 different diseases, each characterised by a specific lysosomal enzyme deficiency in a variety of tissues. They occur in total in about 1 in 5,000 live births and display considerable clinical and biochemical heterogeneity. The majority are inherited as autosomal recessive conditions although two (Hunter disease and Fabry disease) are X-linked. They include Tay-Sachs disease, a gangliosidosis, and

Gaucher's and Niemann-Pick's diseases, which are lipid storage disorders. Most of these diseases affect the brain and are fatal. *(*Brooks et al., Proc Natl Acad Sci USA 2002; 99; 6216-6221*).*

[0016] There has been limited success in only treating the symptoms of these diseases. One way is to replace the enzyme to put a normal gene into the body that can make the enzyme by bone marrow or stem cell transplant or gene therapy. Bone marrow transplantation (BMT) has been successful in several LSDs and allowed long term survival with less severe symptoms. Enzyme replacement therapy (ERT) has been available for patients with Gaucher disease for over 10 years and has provided enormous benefit. However, in many incidents the neurons are unable to take up the large enzyme efficiently even when it is placed next to the cell, so the treatment is still ineffective. It is expected that the stimulation of neuroregeneration and the protection of neurons from apoptosis can at least alleviate the neuronal symptoms and the progress of such lysosomal storage diseases or have a prophylactic effect.

[0017] Multiple sclerosis (MS) is the prototype inflammatory autoimmune disorder of the central nervous system and, with a lifetime risk of one in 400, potentially the most common cause of neurological disability in young adults. Worldwide, there are about 2-5 million patients suffering from this disease *(*Compston & Coles, Lancet 2002, 359: 1221-1231*).* As with all complex traits, the disorder results from interplay between as yet unidentified environmental factors and susceptibility genes. Together, these factors trigger a cascade of events, involving engagement of the immune system, acute inflammatory injury of axons and glia, recovery of function and structural repair, post-inflammatory gliosis, and neurodegeneration. The sequential involvement of these processes underlies the clinical course characterized by episodes with recovery, episodes leaving persistent deficits, and secondary progression. The aim of treatment is to reduce the frequency, and limit the lasting effects of relapses, relieve symptoms, prevent disability arising from disease progression, and promote tissue repair.

[0018] Recently, neuroprotection has been proclaimed an important goal for MS therapy. The basis for widening the scope of neuroprotection is evidence that neuronal and axonal injury are key features of MS lesions. Axon loss most likely determines the persistent neurological deficit in progressive MS. Recent studies pointed out that axon damage occurs early in the disease and during lesion development. Two different phases of axon degeneration were characterized, the first occurring during active myelin breakdown and the second in chronic demyelinated plaques in which the naked axon seems more susceptible to further damage. In contrast with degenerative and ischaemic central nervous system injury, however, neurodegeneration in MS appears to be caused by an inflammatory, presumably autoimmune, process. The challenge for neuroprotection in MS is therefore greater than in degenerative and ischaemic disorders, because MS requires the combination of neuroprotective therapy and effective immunomodulation. The exact mechanisms and effector molecules of axonal degeneration, however, are not vet defined, and an axon-protective therapy has not yet been established. (Bruck & Stadelmann, Neurol Sci. 2003; 24: S26S-S267; Hohlfeld, Int MS J 2003; 10: 103-105).

[0019] Spinal cord injury (SCI) occurs when a traumatic event results in damage to cells within the spinal cord or severs the nerve tracts that relay signals up and down the spinal cord. The most common types of SCI include contusion (bruising of the spinal cord) and compression (caused by pressure on the spinal cord). Other types of injuries include lacerations (severing or tearing of some nerve fibers, such as damage caused by a gun shot wound), and central cord syndrome (specific damage to the corticospinal tracts of the cervical region of the spinal cord). Severe SCI often causes paralysis (loss of control over voluntary movement and muscles of the body) and loss of sensation and reflex function below the point of injury, including autonomic activity such as breathing and other activities such as bowel and bladder control. Other symptoms such as pain or sensitivity to stimuli, muscle spasms, and sexual dysfunction may develop over time. SCI patients are also prone to develop secondary medical problems, such as bladder infections, lung infections, and bed sores. While recent advances in emergency care and rehabilitation allow many SCI patients to survive, methods for reducing the extent of injury and for restoring function are still limited. Immediate treatment for acute SCI includes techniques to relieve cord compression, prompt (within 8 hours of the injury) drug therapy with corticosteroids such as methylprednisolone to minimize cell damage, and stabilization of the vertebrae of the spine to prevent further injury. The types of disability associated with SCI vary greatly depending on the severity of the injury, the segment of the spinal cord at which the injury occurs, and which nerve fibers are damaged. It has long been believed that the adult mammalian central nervous system does not regenerate after SCI. However, cell replacement has been observed recently as an extensive natural compensatory response to injury in the primate spinal cord that contributes to neural repair and is a potential target for therapeutic enhancement *(*Yang et al., J Neurosci. 2006; 26: 2157-2166*).* Advances in the field of stem cell biology, including the identification neural stem cells, has provided new insight for the development of novel therapeutic strategies aimed at inducing regeneration In the damaged central nervous system , i.e. the activation of endogenous these neural stem cells *(*Okano, Ernst Schering Res Found Workshop. 2006; 60:215-228*).*

[0020] Dementia is the progressive decline in cognitive function due to damage or disease in the brain beyond what might be expected from normal aging. Particularly affected areas may be memory, attention, language, and problem solving. Especially in the later stages of the condition, affected persons may be disoriented in time, in place, and in person. For the majority of dementia subtypes there is no cure to this illness, although scientists are progressing in making a type of medication that will slow down the process. Cholinesterase inhibitors are often used early in the disease course. Cognitive and behavioral interventions may also be appropriate. Memantine within the class known as N-methyl-

D-espartate (NMDA) blockers has been approved by the FDA for the treatment of moderate-to-severe dementia. Impaired neurogenesis plays an important role for the decline of cognitive function, e.g. memory, during dementia. It has been shown that recovery of neuronal cell production was associated with the ability to acquire trace memories. The results indicate that newly generated neurons in the adult participate in the formation of a hippocampal-dependent memory (Shors et al., Nature 2001; 410:372-376). Neurosciences have shown that there is a biology of cognition and that neurogenesis and apoptosis are permanently in confrontation in the brain. The present targets of pharmacology are directed against the decline of the neurone and of cognitive performance (Allain et al., Psychol Neuropsychiatr Vieil. 2003; 1:151 - 156).

[0021] Schizophrenia is one of the most common mental illnesses. About 1 of every 100 people (1% of the population) is affected by schizophrenia. This disorder is found throughout the world and in all races and cultures. Schizophrenia affects men and women In equal numbers, although on average, men appear to develop schizophrenia earlier than women. Generally, men show the first signs of schizophrenia in their mid 20s and women show the first signs in their late 20s. Schizophrenia has a tremendous cost to society, estimated at $32.5 billion per year in the US. Schizophrenia is characterized by several of the following symptoms: delusions, hallucinations, disorganized thinking and speech, negative symptoms (social withdrawal, absence of emotion and expression, reduced energy, motivation and activity), catatonia. The main therapy for schizophrenia is based on neuroleptics, such as chlorpromazine, haloperidol, olanzapine, clozapine, thioridazine, and others. However, neuroleptic treatment often does not reduce all of the symptoms of schizophrenia. Moreover, antipsychotic treatment can have severe side effects, such as tardive dyskinesias. The etiology of schizophrenia is not clear, although there seems to be a strong genetic Influence. Recently, it has become clear that schizophrenia has at least some aspects of a neurodegenerative disease. In particular, MR studies have revealed rapid .cortical grey matter loss in schizophrenic patients (Thompson et al., Proc Natl Acad Sci USA 2001; 98: 11650-11655; Cannon et al., Proc Nat] Acad Sci USA 2002; 99: 3228-3233). Therefore, treatment of schizophrenics with neuroprotective and/or neuroregenerative medication is warranted.

[0022] Peripheral neuropathy is a pain initiated or caused by a primary lesion or dysfunction of the nervous system. Many classification systems exist but typically it is divided into central (i.e. thalamic, post-stroke pain) and peripheral deafferent pain (i.e. meralgia paresthetica). Neuropathies may affect just one nerve (mononeuropathy) or several nerves (polyneuropathy). They are allodynia, hyperalgesia, and dysesthesias. Common symptoms include burning, stabbing, electric shock, or deep aching sensations. The causes of neuralgia include diabetic neuropathy, trigeminal neuralgia, complex regional pain syndrome and post-herpetic neuralgia, uremia, AIDS, or nutritional deficiencies. Other causes include mechanical pressure such as compression or entrapment, direct trauma, penetrating injuries, contusions, fracture or dislocated bones; pressure involving the superficial nerves (ulna, radial, or peroneal) which can result from prolonged use of crutches or staying in one position for too long, or from a tumor; intraneural hemorrhage; exposure to cold or radiation or, rarely, certain medicines or toxic substances; and vascular or collagen disorders such as atherosclerosis, systemic lupus erythematosus, scleroderma, sarcoidosis, rheumatoid arthritis, and polyarteritis nodosa. A common example of entrapment neuropathy is carpal tunnel syndrome, which has become more common because of the increasing use of computers. Although the causes of peripheral neuropathy are diverse, they produce common symptoms including weakness, numbness, paresthesia (abnormal sensations such as burning, tickling, pricking or tingling) and pain in the arms, hands, legs and/or feet. A large number of cases are of unknown cause.

[0023] Treating the underlying condition may relieve some cases of peripheral neuropathy. In other cases, treatment may focus nn managing pain. Therapy for peripheral neuropathy differs depending on the cause. For example, therapy for peripheral neuropathy caused by diabetes involves control of the diabetes. In cases where a tumor or ruptured disc is the cause, therapy may involve surgery to remove the tumor or to repair the ruptured disc. In entrapment or compression neuropathy treatment may consist of splinting or surgical decompression of the ulnar or median nerves. Peroneal and radial compression neuropathies may require avoidance of pressure. Physical therapy and/or splints may be useful in preventing contractures. Peripheral nerves have a remarkable ability to regenerate themselves, and new treatments using nerve growth factors or gene therapy may offer even better chances for recovery in the future. Therefore, the enhancement of this regeneration of the peripheral nerves by neuroregenerative medication is warranted.

[0024] In humans there is a need for ways to increase cognitive capacities, and boost intelligence. "Intelligence" in modern understanding is not limited to purely logical or semantic capabilities. For example, the theory of multiple intelligences by Howard Gardner evaluates intelligence from evolutionary and anthropological perspectives and yields a broader view that includes athletic, musical, artistic, and empathetic capacities as well as the linguistic/logical abilities that are more commonly associated with intelligence and measured by IQ tests. This broader sense of intelligence also extends into the area nf creativity. In addition, there is a non-pathological condition known in the human as ARML (age-related memory loss) or MCI (mild cognitive impairment) or ARCD (age-related cognitive decline) that usually commences at about age 40, and is different from early signs of Alzheimer's disease.

[0025] There is a physiological loss of nerve cells throughout adulthood, estimated to as many as 100,000 neurons a day. Throughout adulthood, there is a gradual reduction in the weight and volume of the brain. This decline is about 2% per decade. Contrary to previously held beliefs, the decline does not accelerate after the age of 50, but continues

at about the same pace from early adulthood on. The accumulative effects of this are generally not noticed until older age.

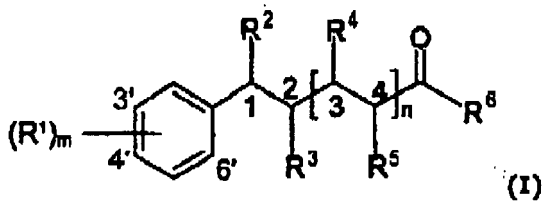
[0026] While the brain does shrink in size, it does not do so uniformly. Certain structures are more prone to shrinkage. For example, the hippocampus and the frontal lobes, two structures Involved in memory, often become smaller. This is partly due to a loss of neurons and partly due to the atrophy of some neurons. Many other brain structures suffer no loss in size. The slowing of mental processing may be caused by the deterioration of neurons, whether they are lost, shrink, or lose connections. This depletion of fully functioning neurons makes It necessary to recruit additional networks of neurons to manage mental tasks that would otherwise be simple or automatic. Thus, the process is slowed down.

[0027] A portion of the frontal lobe, called the prefrontal cortex, is involved in monitoring and controlling thoughts and actions. The atrophy that occurs in this brain region may account for the word finding difficulties many older adults experience. It may also account for forgetting where the car keys were put or general absentmindedness. The shrinkage of both the frontal lobe and the hippocampus are thought to be responsible for memory difficulties. Therefore, there also remains a need for improving or enhancing the cognitive ability of an individual. This may be achieved by the enhancement of neuroprotective and/or neuroregenerative processes of the brain.

[0028] In view of the above, there is a need for treating neurological and/or psychiatric conditions, such as neurological diseases that relate to the enhancement of plasticity and functional recovery, or cell-death in the nervous system. In particular, there is a need for treating neurological diseases by providing neuroprotection to the neural cells involved or to induce neurogenesis to recover from neuronal loss.

[0029] Accordingly, the technical problem underlying the present invention may be seen as the provision of means and methods for treating, ameliorating and/or preventing neurological and/or psychiatric conditions caused by neurodegeneration or impaired or insufficient neurogenesis. The technical problem is solved by the embodiments characterized in the claims and herein below.

[0030] Accordingly, the present invention relates to the use of a compound with the general formula (I)



(I)

wherein

n=0, 1, or 2, provided that

when n=0, R^2 and R^3 represent hydrogen atoms or together represent a carbon to carbon double bond, either in E or in Z geometric configuration;
when n=1, or 2, R^2 and R^3 represent hydrogen atoms or together represent a carbon to carbon double bond, either in E or in Z geometric configuration, and R^4 and R^5 represent hydrogen atoms or together represent a carbon to carbon double bond, either in E or in Z geometric configuration;

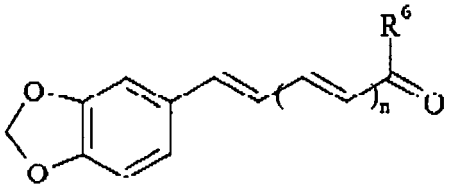
m=0, 1, 2, or 3, provided that

when m=1, R^1 represents an alkoxy group having, from 1 to 3 carbon atoms, or a hydroxy group,
when m=2, each R^1 independently represents an alkoxy group having from 1 to 3 carbon atoms or the two R^1 together represent a 3',4'-methylenedioxy group;
when m=3, two R^1 together represent a 3',4'-methylenedioxy group and the other R^1 represents an alkoxy group having from 1 to 3 carbon atoms, or a hydroxy group;

R^b represents a pyrrolidino, piperidino, 4-methylpiperidino, morpholino, N-monoalkylamino group of 4 to 6 carbon atoms, N-monocycloalkylamino group of 4 to 7 carbon atoms, 3',4'-methylenedioxy-substituted benzylamino group, 2-phenethylamino group, or an alkoxy group having from 1 to 6 carbon atoms

for the preparation of a pharmaceutical composition for treating and/or preventing a neuronal condition.

[0031] Preferred compounds of the present invention have the general formula (II)



(II)

wherein

n is 0 or 1 and R^6 has the meaning as indicated above.

[0032] Preferably, R^6 represents a pyrrolidino, piperidino, 4-methylpiperidino, morpholino, N-monoalkylamino group of 4 to 6 carbon atoms, N-monocycloalkylamino group of 4 to 7 carbon atoms, 3',4'-methylenedioxy-substituted benzylamino group, or 2-phenethylamino group.

[0033] The general formulas I and II represent piperine and derivatives thereof.

[0034] More preferably, the compound of the present invention is piperine, Trichostachine, Piperlonguminine, 5-E,E-piperlnoyl methylamine, 5-C,C-piperinoyl ethylamine, 5-E,E-piperinoyl isopropylamine, 5-E,E-piperinoyl cyclohexylamine, 5-E,E-piperinoyl butylamine, Despiperidyl-methoxypiperidine, 5-E,E-piperinoyl morpholine, 5-E,E-piperinoyl hexylamine, 5-E,E-piperinoyl piperinoylamine, 5-E,E-piperinic acid ethyl ester, 5-E,E-piperinic acid isopropyl ester, 5-E,E-piperinic acid propyl ester, 5-E,E-piperinic acid butyl ester, Antiepilepsirine, 1-(3-trans-benzo-1,3-dioxol-5-ylacryloyl) cyclohexylamine, 1-(3-trans-benzo-1,3-dioxol-5-ylacryloyl) cycloheptylamine, 1-(3-trans-benzo-1,3-dioxol-5-ylacryloyl) cyclopenylamine, 1-(3-trans-benzo-1,3-dioxol-5-ylacryloyl) pyrrolidine, 1-(3-trans-benzo-1,3-dioxol-5-ylacryloyl) morpholine, 3-trans-benzo-1,3-dioxol-5-ylacrylic acid methyl ester, Piperettine, Coumaperine, 4'-Methoxyiso-coumaperine, Wisanine, 1-(4-methoxy-cinnamoyl)piperldine, 1-(3-methoxy-cinnamoyl)piperidine, 1-(2-methoxy-cinnamoyl)piperidine, 1-cinnamoyl-piperidine, 1-(3,4-dimethoxy-cinnamoyl)piperidine, 3-benzo-1,3-dioxol-5-ylpropionic acid piperidide, 1,2,3,4-Tetrahydropiperine, Piperanine, Chavicine, Isoplperine, or Isochavicine.

[0035] In particular, the compound of the present invention is piperine.

[0036] These compounds are known in the art and listed in the table 1 below indicating the chemical name, the definition of the variables of formula (I) and the references, where the compounds are cited.

Table 1

name	n	m-R1	R6	reference.
Piperine	n=1; R2 and R3 form carbon to carbon double bond in E configuration; R4 and R5 form carbon to carbon double bond in E configuration	m=2; R1 represents 3',4'-methylenedioxy group	R6 represents piperidino	US6,346,539
Trichostachine (RV-A01)			group R6 represents pyrrolidino group	
Piperlonguminine (RV-A06)			R6 represents isobutylamino group	
5-E,E-piperinoyl methylamine (RV-A07)			R6 represents methylamine group.	
5 E,E piperinoyl ethylamine (RV-A08)			R6 represents ethylamino group	
5-E,E-piperinoyl isopropylamine (RV-A09)			R6 represents isopropylamino group	
5-E,E-piperinoyl cyclohexylamine (RV-A10)			R6 represents cyclohexylamino group	
5-E,E-piperinoyl butylamine (RV-A11)			R6 represents butylamino group	
Despiperidyl-methoxypiperidine (RV-AB1)			R6 represents methoxy group	
5-E,E-piperinoyl morpholine (RV-A02)			R6 represents morpholino group	
5-E,E-piperinoyl hexylamine (RV-A05),			R6 represents hexylamino group	
5-E,E-piperinoyl piperinoylamine (RV-A04)			R6 represents 3',4'-methylenedioxy-benzylamino group	
5-E,E-piperinic acid ethyl ester (RV-AB2)			R6 represents ethoxy group	
5 E,E piperinic acid isopropyl ester (RV-AB4)			R6 represents Isopropoxy group	
5-E,E-piperinic acid propyl ester (RV-AB5)			R6 represents propoxy group	
5-F,E-piperinic acid butyl ester (RV-AB6)			R6 represents butoxy group	

(continued)

name	n	m-R1	R6	reference.
Antlepilepsirine; Ilepcimide; 1-(3-trans-benzo-1,3-dioxol-5-ylacryloyl) piperidine (RV-B01)	n=0, R2 and R3 form Carbon to carbon double bond in E configuration	m=2; R1 represents 3',4'-methylenedioxy group	R6 represents piperidino group	Pei-1983; Liu-1984; US6,346,539
1-(3-trans-benzo-1,3-dioxol-5-ylacryloyl) cyclohexylamine			R6 represents cyclohexylamino group	ChemBridge Corporation
1-(3-trans-benzo-1,3-dioxol-5-ylacryloyl) cycloheptylamine			R6 represents cycloheptylamino group	
1-(3-trans-benzo-1,3-dioxol-5-ylacryloyl) cyclopentylamine			R6 represents cyclopentylamino group	
1-(3-trans-benzo-1,3 dioxol-5-ylacryloyl) pyrrolidine (RV-B02)			R6 represents pyrrolidino group	ChemBridge Corporation; U56,346,539
1-(3-trans-benzo-1,3-dioxol-5-ylacryloyl) morpholine (RV-B03)			R6 represents morpholino group	
3-trans-benza-1,3-dioxol-5-ylacrylic acid methyl esther (RV-BB1)			R6 represents methoxy group	

(continued)

name	n	m-R1	R6	reference.
Piperettine	n=2; R2 and R3 form carbon to carbon double bond; R4 and R5 form carbon to carbon double bond; each double bond in E configuration		R6 represent piperidlno group	US6,346,539
Coumaperine	n=1; R2 and R3 form carbon to carbon double bond in E configuration; R4 and R5 form Carbon to carbon double bond in E configuration	m=1; R1 represents 4'-hydroxy group		
4'-Methoxyiso-coumaperine		m=2; R1 represent 3'-hydroxy group and 4'methoxy group		
Wisanine		m=3; R1 represent 3',4'-methylenedioxy group and 6'-methoxy group methoxy group		
1-(4-methoxy-cinnamoyl) piperidine (RV-G03)	n=0; R2 and R3 form carbon to carbon double bond In E configuration	m=1; R1 represents 4'-methoxy group	R6 represents piperidino group	US6,346,539
1-(2-methoxy-cinnamoyl) piperidine (RV C01)		m=1; R1 represents 6'-methoxy group		
1-(3-methoxy-cinnamoyl) piperidine (RV-G02)		m=1; R1 represents 3'-methoxy group		
1-cinnamoyl-piperidine (7306)		m=0		Pei-1983
1-(3,4-dimethoxy-cinnamoyl) piperidine (RV-G04)		m=2; R1 represent 3'-methoxy group and 4'-methoxy group		US6,346,539
3-benzo-1,3-dioxol-5-ylproplonic acid piperldide (RV-C03)	n=0; R2 and R3 represent hydrogen atoms			
1,2,3,4-Tetrahydropiperine (RV-C02)	n=1; R2, R3, R4, and R5 represent hydrogen atoms			

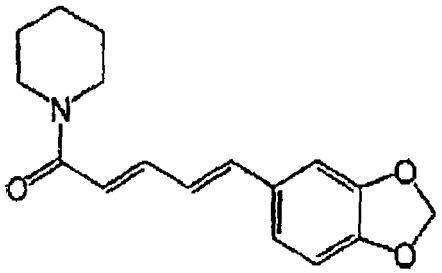
(continued)

name	n	m-R1	R6	reference.
Piperanine/ 3,4,-dihydropiperine	n-1; R2 and R3 form carbon to carbon double bond in E configuration: R4 and R5 represent hydrogen atoms and	m=2; R1 represents 3',4'-methylenedioxy group	R6 represents piperidino group	U56,346,539
Chavicine	n=2; R2 and R3 form carbon to carbon double bond; R4 and R5 form carbon to carbon double bond; each double bond in Z configuration			
Isopiperine	n=2; R2 and R3 form carbon to carbon double bond in Z configuration; R4 and R5 form carbon to carbon double bond in E configuration			
Isochavicine	n=2; R2 and R3 form carbon to carbon double bond in E configuration; R4 and R5 form carbon to carbon double bond in Z configuration			

[0037] The aforementioned compounds to be applied in the uses and methods of the present invention, preferably, retain at least 10%, more preferably 50% of the neuroregenerative and/or neuroprotectve activity of piperine as measured by the in vitro assays described herein.

[0038] Piperine is an alkaloid found naturally in plants belonging to the *Piperaceae* family, such as *Piper nigrum,* commonly known as black pepper, and *Piper longum,* commonly known as long pepper. Piperine is the major pungent substance in these plants and is isolated from the fruit of the black pepper and long pepper plants. The term black pepper is used both for the plant *Piper nigrum* and the spice that is mainly in the fruit of the plant.

[0039] Piperine is a solid substance essentially insoluble in water. It is a weak base that is tasteless at first, but leaves a burning aftertaste. Piperine belongs to the vanilloid family of compounds, a family that also includes capsaicin, the pungent substance in hot chili peppers, Its molecular formula is $C_{17}H_{19}NO_3$, and its molecular weight is 285.34 daltons. Piperine is the trans-trans stereoisomer of 1-piperoylpiperidine. It is also known as (*E*, *E*) 1 piperoylpiperidine and (*F, F*)-1-[5-(1, 3-benzodioxol-5-yl)-1-oxa-2, 4-pentadienyl] piperidine. It is represented by the following chemical structure:



[0040] Piperine was first obtained by Oersted, of Copenhagen, in 1819, who believed it to be an organic base. It may be isolated by various methods. According to Cazeneuve and Caillot (Jahresb. der Pharm., 1877, p. 68), powdered pepper is mixed with milk of lime, the mixture evaporated to dryness on the water-bath, and extracted with ether. This solvent upon evaporation leaves piperine in the form of impure crystals, which are purified best by crystallization from acetone *(Flückiger, 1891*). Sumatra pepper yielded on an average 8.10 %, Singapore white pepper 9.15 % of piperine. Stevenson prepared an extract from 50 g of pepper with methyl alcohol and dissolves out the resinous portion by means of potassium carbonate *(*Stevenson, Amer. Jour. Pharm., 1885, p. 513*).* The residual piperine has been washed with water and recrystallized from alcohol.

[0041] The U.S. Pat. No. 5,744,161 describes the isolation of piperine from a suitable oleoresin material obtained from the fruit or plant nf the Piperaceae family. Isourea, urea or a urea derivative can be used to remove organic matter other than piperine from the oleoresin and thereby to obtain high purity piperine *(*US 6,054,585*).* Extraction of piperine using aqueous hydrotrope solutions is described in the U.S. Pat. No. 6,365,601.

[0042] Black pepper and long pepper have been used in Ayurvedic medicine for the treatment of various diseases. One such preparation Is known by the Sanskrit name trikatu and consists of black pepper, long pepper and ginger. Another preparation, known by the Sanskrit name pippali, consists of long pepper. It is thought that piperine is one or the major bioactive substances of these Ayurvedic remedies.

[0043] Piperine increases thermogenesis and in turn creates a demand for nutrients necessary for metabolism.

[0044] It has putative anti-inflammatory activity and may have activity in promoting digestive processes. The mechanism of piperine's putative anti-inflammatory activity may be accounted for, in part, by piperine's possible antioxidant activity.

[0045] Piperine exhibited significant anti-inflammatory activity in carageenan-induced rat paw edema and in some other experimental models of inflammation. In one animal study, piperine reduced liver lipid peroxidation, acid phosphatase and edema induced by carageenan *(*Dhuley et al., Indian J Exp Biol. 1993; 31:443-445*;* Mujumdar et al., Jpn J Med Sci Biol. 1990; 433:95-100).

[0046] In a rat intestinal model, piperine was said to provide protection against oxidative changes induced by a number of chemical carcinogens (Khajuria et al., Mol Cell Biochem. 1998; 189:113-118)

[0047] There are *in vitro,* animal and human studies demonstrating that piperine can significantly increase the bioavailability of numerous drugs and some nutritional supplements (Atal et al., J Pharmacol Exp Ther. 1985; 232:258-262*;* Badmaev et al. Nutr Res. 1999; 19:381-388*;* Badmaev et al. J Nutr Biochem. 2000; 11:109-113*;* Bano et al., Eur J Clin Pharmacol. 1991; 41:615-617*;* Pattanaik et al., Phytother Res. 2006; 20:683-686*;* US5616593*;* US 5,972,382*;* US 6,017,932*;* EP0650728*;* EP1494749*).* Reportedly, It has demonstrated this effect with some antimitrobial, antiprotozoal, antihelmintic, antihistaminic, non-steroidal anti-inflammatory, muscle-relaxant and anticancer drugs, among others. It has also increased the bioavailability of coenzyme Q_{10}, curcumin, beta-carotene, propanolol and theophylline.

[0048] The mechanism is thought to be by inhibition of certain enzymes involved in the biotransformation of the affected drugs. Piperine has been found to be a non specific inhibitor of drug and xenobiotic metabolism. It appears to inhibit many different cytochrome P450 isoforms, as well as UDP-glucuronyltransferase and hepatic arylhydrocarbon hydroxylase and other enzymes involved in drug and xenobiotic metabolism. However, it is not yet possible to predict on theoretical grounds the effects piperine will have on any chosen dietary substance or drug.

[0049] Aside from its effects on bioavailability, piperine has a number of various other actions in the body such as stimulating the production of beta-endorphins, serotonin, adrenaline, melanin and digestive enzymes, relieving asthma symptoms and pain, and reducing ulceration and production of acid of the stomach.

[0050] Piperine and derivatives thereof as represented by the general formula I have also been demonstrated to stimulate the proliferation of melanocytes. Therefore, pharmaceutical preparations of these compounds were suggested for the treating of skin pigmentation disorders (EP1094813).

[0051] Black pepper has also been used in traditional Chinese medicine to treat seizure disorders. Piperine and derivatives thereof such as antiepilepsirine, have been claimed to have some anticonvulsant activity and therefore have been used In China to treat some forms of epilepsy *(*Pei, Epilepsia 1983; 24:177-182; Liu et al., Biochemical Pharmacology

1984; 33:3883-3886).

[0052] In mice, piperine injected Intra-peritoneally inhibited clonic convulsions induced by kainate. It did not significantly block seizure activity induced by L-glutamate, N-methyl-D-aspartate or guanidinosuccinate (D'Hooge et al., Arzneimitteltorschung. 1996; 46:557-560).

[0053] The pharmacokinetics of piperine in humans remains incompletely understood. In rats, piperine is absorbed following ingestion, and some metabolites have been identified: piperonylic acid, piperonyl alcohol, piperonal and vanillic acid are found in the urine. One metabolite, piperic acid, is found in the bile. Piperine is absorbed quickly and well from the digestive tract. Effects on absorption of other substances begin around 15 minutes after dosing and last for an hour or two. Blood levels peak about 1-2 hours after dosing but effects on metabolic enzymes can last much longer. Further human pharmacokinetic studies are needed.

[0054] Piperine is structurally related to capsaicin and zingerone which are also natural pungent-tasting compounds found in chili pepper and ginger, respectively. These compounds having a vanillyl moiety in common are capable to agonize to the vanilloid receptors which can be inhibited by Capsazepine (Liu & Simon et al., J Neurophysiol. 1996; 76: 1858-1869). Piperine has been found to predominantly activate the vanilloid receptor subtype TRPV1 which can be inhibited selectively and potently by the substance SB-366791 (McNamara et al., Br J Pharmacology 2005; 144:781-790; Varga et al., Neurosci Lett. 2005; 385:137-142; Gunthrope et al., Neuropharmacology 2004; 46:133-146).

[0055] Derivatives of piperine which comprise minor variations of the formula usually exhibit basically unchanged biological effects. Therefore, they can be used alternatively to piperine. Various derivatives of piperine sharing its functional activities as well as the syntheses and the characterizations thereof are known by the literature (EP1094813; Pel, Epilepsia 1983; 24:177-182; Liu et al., Biochemical Pharmacology 1984; 33:3883-3886).

[0056] Methods for isolation and/or synthesis of compounds to be used in accordance with the present invention are known by the literature (US 5,744,161; US 6,054,585; US 6,365,601; EP1094813; US 6,346,539; Pei, Epilepsia 1983; 24:177-182; Liu et al., Biochemical Pharmacology 1984; 33:3883-3886). Minor chemical variations of piperine can potentially improve physical and/or biological properties such as stability, solubility, ability to penetrate the blood-brain-barrier without erase piperine's neuroregenerative and/or neuroprotective effect. It is a well established method for the person skilled in the art to modify the initial pharmacologically active compound in order to improve e.g. the pharmacokinetics or the profile of adverse side effects. The in vitro and in vivo assays described herein are suitable to test the neuroregenerative and neuroprotective effect of such modified compounds.

[0057] Piperine as well as the structurally related compounds capsalcin and zingerone which have the vanillyl moiety in common are known agonists of the TRPV1 receptor (Liu & Simon et al., J Neurophysiol. 1996; 76:1858-1869). However, it has been found in accordance with the present invention that the neuroregenerative and the neuroprotective effect of piperine is not mediated by the agonization of this receptor. There is no detectable neuroregenerative and neuroprotective effect with capsaicin in the in vitro assays described herein. Furthermore, the neuroregenerative and neuroprotective effect of piperine in the in vitro assays described herein is not diminished hy the specific TRPV1 receptor inhibitor SB366791.

[0058] A more detailed specification of these findings is given in Example 6.

[0059] The term "neurogenesis" as used herein refers, in principle, to the formation of neurons from stem cells. Recently, the importance of forming new nerve cells (neurogenesis) for treating neurological disease has been recognized, Unlike many other tissues, the mature brain has limited regenerative capacity, and its unusual degree of cellular specialization restricts the extent to which residual healthy tissue can assume the function of damaged brain. However, cerebral neurons are derived from precursor cells that persist in the adult brain, so stimulation of endogenous neural precursors in the adult brain could have therapeutic potential.

[0060] Neurogenesis bases on the differentiation of neuronal stem cells to new neurons. It occurs in discrete regions of the adult brain, including the rostral subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus (DG). Neurogenesis occurs in the adult animal especially after a particular neurological paradigm - e.g. cerebral ischemia (Jin et al., Proc. Natl. Acad. Sci. USA 2001; 98: 4710-4715; Jiang et al., Stroke 2001; 32: 1201 -1207 ; Kee et al., Exp. Brain. Res. 2001; 136; 313-320,; Perfilieva et al., J- Cereb. Blood Flow Metab. 2001; 21: 211-217). Neurogenesis has also been demonstrated in humans (Eriksson et al., Nat Med. 1998; 4: 1313-1317), and indeed leads to functional neurons (van Praag et al., Nature 2002; 415: 1030-1034). In particular, the subgranular zone of the dentate gyrus, and the hilus has the potential to generate new neurons from adult neuronal stem cells (Gage et al., J Neurobiol 1998; 36: 249-266).

[0061] Adult neuronal stem cells do not possess the ability to differentiate into all cell types of the organism (totipotent) as embryonal stem cells do, but they have the potential to differentiate to the various cell types of the brain tissue (pluripotent). During the differentiation they undergo essential morphological and functional changes (van Praag et al., Nature 2002; 415: 1030-1034). The regeneration of neuronal cells (neuroregeneration) depends on the mechanisms of neurogenesis and allows for the recovery of neurodegenerative processes. Compounds which possess the ability to induce neurogenesis can potentially be used in more than one disorder, as the necessity of regeneration of neuronal tissue (in both acute disorders and in chronic neurodegenerative diseases) is similar.

[0062] The term "neuroprotection" means mechanisms within the nervous system which protect neurons from apoptosis or degeneration, for example following a brain injury or as a result of chronic neurodegenerative diseases. The goal of neuroprotection is to limit neuronal dysfunction/death after CNS injury and attempt to maintain the highest possible integrity of cellular interactions in the brain resulting in an undisturbed neural function. I here is a wide range of neuroprotection products under investigation (e.g. free radical scavengers, Apoptosis inhibitors, neurotrophic factors, metal ion chelators, and anti-excitotoxic agents). Neuroprotection products can potentially be used In more than one disorder, as many of the underlying mechanisms of damage to neural tissues (in both acute disorders and in chronic neurodegenerative diseases) are similar.

[0063] Advantageously, a neuroregenerative acting compound, such as piperine and derivatives thereof, allows for the treating of a neurological condition even at a late stage after progressive neuronal loss. Such a compound enables to reverse neuronal loss which have had already occurred. By contrast a compound which solely acts neuroprotective can be used only to stop or attenuate the ongoing process of neuronal dieing.

[0064] Specifically, it has been unexpectedly found that the compounds referred to in accordance with the present invention have a neuroprotective and neuroregenerative effect. Therefore, these compounds can be used alone or in combination tor the treatment of neurological conditions or diseases where there is a need of neuroprotection and/or neuroregeneration.

[0065] Thus, contemplated by the present invention is also a method of treating a patient with a neurological condition comprising administering in a therapeutically effective amount a compound as defined above to a patient suffering from the said neurological condition. Preferred neurological conditions or diseases to be treated are those referred to elsewhere in this specification explicitly.

[0066] It has been shown that the compounds according to the present invention have the ability to enhance neurogenesis, and therefore e.g. improve behavioural outcome after an ischemic lesion. Neurogenesis is one mechanism that can lead to increased plasticity of neural networks, and can replace gradual loss of neurons. Therefore, one embodiment of the present invention is to provide enhancement, improvement or an increase in cognitive ability to an individual suffering from, displaying, and/or believed to some level of cognitive loss by administering one or more compositions as described herein to the individual in accordance with the administration discussion herein. In an alternative embodiment, cognitive enhancement may also benefit those individuals even useful under non-pathological conditions, e.g., those individuals who do not present with cognitive impairment.

[0067] Further to the neuroregenerative activity, it has been found that the compounds act anti-apoptotic on neuronal cells and thereby they can enhance the viability of neurons. Therefore the compounds referred to in accordance with the present invention can be regarded as neuroprotective compounds. The term "neuroprotective compounds" refers to substances which have a protective effect on neural cells and/or the brain tissue in total either in vitro (cell culture systems) or in vivo (animal models for ischemic, hypoxic and/or neurodegenerative diseases, such as stroke, ALS or Huntington's disease) or in human patients.

[0068] The neuroregenerative effect of the compounds according to the present invention can be assayed in vitro hy measuring their stimulation of the differentiation of neuronal stem cells. Therefore, adult neuronal stem cells have to be isolated and subsequently cultivated in a suitable culture medium. After several passages in vitro the stem cells have to be co-transfected with a DNA construct coding for two discriminable reporter proteins one of them under the control of a constitutive promoter and the other under the control of a beta -III-tubulin promoter. The beta-III-tubulin promoter is induced during the process of neuronal stem cell differentiation; therefore the reporter protein under the control of this promoter serves as a marker for the stem cell differentiation in such an in vitro dual reporter assay *(*Schneider et al., J Clin Investigation 2005; 115:2083-2098*).* The person skilled in the art knows several suitable constitutive promoters such as the SV40-promoter, the TK-promoter, or the CMV-promoter. As discriminable reporter proteins for such a dual reporter assay one can use discriminable luciferases such as firefly luciferase and Renilla luciferase. The transfected cells have to be incubated with the test compound and with a positive and negative controls for normalization and background correction. As a positive control compound one can use retinoic acid *(*Guan et al., Cell Tissue Res. 2001; 305:171-176*;* Jacobs et al., Proc. Natl. Acad. Sci. USA 2006; 103:3902-3907*),* a suitable negative control is the sham treatment of the cells. The neroregenerative activity of the test compound is determined as the ratio of the signal of the beta-III-tubulin promoter controlled reporter versus the signal of the constitutively expressed reporter and is expressed as fold from the control. This assay is suitable to assess the neuroregenerative activity of piperine and derivatives thereof, but also can be used to screen for other potential neuroregenerative compounds. Preferably, such a screening can be used to test compounds which are already known to be suitable as a medicament in terms of its toxicology, bioavailability, solubility, stability etc.

[0069] A more detailed specification of such an in vitro assay for the assessment of neuroregenerative effect is given in Examples 1 and 2.

[0070] Neuroprotection is attainable via the inhibition of apoptosis of affected neurons. The anti-apoptotic effect of piperine and its derivatives can be assayed in vitro by measuring the caspase-3 and caspase 7 activities. These members of the cysteine aspartic acid specific protease (caspase) family play key effector roles in apoptosis in mammalian cells.

Cortical neurons have to be isolated and subsequently cultivated in a suitable culture medium. The cells have to be incubated with the test compound in the presence of an apoptotic substance such as staurosporine. Parallel approaches with staurosporine alone and sham treatment of the cells serve as negative and positive controls. The assay allows evaluating whether the test compound can compensate either completely or partially for the apoptotic effect of staurosporine. This assay is suitable to assess the neuroprotective activity of piperine and derivatives thereof, but also can be used to screen for other potential neuroprotective compounds. Preferably, such a screening can be used to test compounds which are already known to be suitable as a medicament in terms of its toxicology, bioavailability, solubility, stability etc.

[0071] A more detailed specification of such an in vitro assay for the assessment of an anti-apoptotic effect on neurons is given in Examples 3 and 4.

[0072] The viability assay allows for further assessment of test compounds regarding their effect on cells such as neurons. Therefore, a stably transfected cell, line (e.g. SH-SYSY cells) with a reporter construct (e.g. a luciferase construct) has to be incubated with the test compound and in parallel with positive and negative controls (e.g. staurosporine and sham, respectively). Subsequently, the signal generated by the reporter construct serves as a readout for the viability of the cells in the presence of the test compound. The assay enables the evaluation of potential neurotoxic effects of the test compound itself. The inventors found that piperine and derivatives thereof have no cytotoxic effect in this assay.

[0073] A more detailed specification of such an in vitro assay for the assessment of an effect on the viability of neurons is given in Example 5.

[0074] Animal models for certain neurological conditions and diseases can be used for the further evaluation of the in vivo effect of a test compound. The rat MCAO model (middle cerebral occlusion; filament model) is an animal model for stroke, where a broad variety of different neuron types is damaged by ischemia/hypoxia. Further animal models for various neurological conditions are well known by the person skilled in the art. Such animal models are for example but not limited to SOD1G93A-transgenic mouse for amyotrophic lateral sclerosis *(*Almer et al., J Neurochem 1999; 72: 2415-2425), APP-transgenic mice for Alzheimer's disease *(*Janus & Westaway, Physiol Behav 2001; 73:873-886*),* exon-1-huntingtin-transgenic mice for Huntington's disease *(*Sathasivam et al., Philos Trans R Soc Lond B Biol Sci. 1999; 354: 963-969), and FRDA-transgenic mice for Friedreich ataxia *(*Al-Mahdawi Genomics 2006:88:580-590*).* The animals treated with the test compound have to be compared with sham treated animals. The inventors found that the treatment of rat MCAO model animals with piperine resulted in a significantly reduced infarct volume compared to the sham treatment. This analysis demonstrates that piperine exhibits neuroprotective activity in vivo in neurological conditions such as stroke. It also can be used to assess for the neuroprotective activity of derivatives of piperine.

[0075] A more detailed specification of an analysis of piperine in an animal model for stroke is given in Example 7.

[0076] The compounds referred to in accordance with the present invention , alone, in combination with each other, and/or in combination with one or more additional ractors can be used to treat neurological conditions with a need for neuroprotection and neuroregeneration. These neurological conditions comprise cerebral ischemia (such as stroke, traumatic brain injury, or cerebral ischemia due to cardiocirculatory arrest), amyotrophic lateral sclerosis, glaucoma, Alzheimer's disease, neurodegenerative trinucleotide repeat disorders (such as Huntington's disease), neurodegenerative lysosomal storage diseases, multiple sclerosis, spinal cord injury, spinal cord trauma, dementia, schizophrenia, and peripheral neuropathy. Additionally, the said compounds, alone, in combination with each other, and/or in combination with one or more additional factors can be used to enhance learning and memory, e.g. in the case of non-pathological conditions such as age-related memory loss, mild cognitive impairment, or age-related cognitive decline,

[0077] In a preferred embodiment of the uses and methods nf the present invention, the compounds, alone, in combination with each other, and/or in combination with one or more additional factors can be used to treat neurological conditions which are based on ischemia and/or hypoxia of the neural tissue such as cerebral ischemia (e.g. stroke, traumatic brain injury, or cerebral ischemia due to cardiocirculatory arrest), glaucoma, spinal cord injury, or spinal cord trauma by providing protection and regeneration neuronal cells in those patients with the condition. The common pathological and protective processes active in these neurological conditions indicate that a therapy the compounds according to the present invention will be comparably effective.

[0078] By "treating" is meant the slowing, interrupting, arresting or stopping of the progression of the disease or condition and does not necessarily require the complete elimination of all disease symptoms and signs. Moreover, as will be understood by those skilled in the art, such treatment is usually not intended to effective for 100% of the subjects to be treated. The term, however, requires that a statistically significant portion of subjects can be efficiently treated, i.e. the symptoms and clinical signs can be ameliorated. Whether a portion is statistically significant can be determined without further ado by the person skilled in the art using various well known statistic evaluation tools, e.g., determination of confidence intervals, p-value determination, Student's ttest, Mann-Whitney test etc.. Preferred confidence intervals are at least 90%, at least 95%, at least 97%, at least 98% or at least 99 %. The p-values are, preferably, 0.1, 0.05, 0.01, 0.005, or 0.0001.

[0079] For certain neurological diseases (e.g. amyotrophic lateral sclerosis and Huntington's disease) a predisposition

can be determined based on genetic markers or familial accumulation of the disease. For other neurological conditions (e.g. cerebral Ischemia such as stroke) various risk factors such as smoking, malnutrition, or certain blood levels are known by the person skilled in art. The treatment of such a neurological disease is most promising when starting very early even before the onset of the clinical symptoms *(*Ludolph, J Neurol 2000; 247:VI/13-VI/18*).* At that stage of the disease the molecular and cellular damage already has begun. It is obvious that a neuroregenerative and/or neuroprotective compound is most effectively preventing the neurological condition or disease when given as soon as the cellular damage (i.e. the loss of neurons) just has started.

[0080] Compounds to be used in accordance with the present invention, such as piperine ,which are well tolerated even upon long-term application can be used as a prophylaxis to treat those patients that are at risk of developing such a neurological conditions or diseases. Prophylactic administration should be administered in a way to establish a sustained level of compound in an effective amount for the stimulation of neurogenesis and for neuroprotection.

[0081] "Preventing" is intended to include the prophylaxis of the neurological disease or condition, wherein "prophylaxis" Is understood to be any degree of inhibition of the time of onset or severity of signs or symptoms of the disease or condition, including, but not limited to, the complete prevention of the disease or condition. Moreover, as will be understood by those skilled in the art, such a prevention is usually not intended to effective for 100% of the subjects to be subjected to it. The term, however, requires that a statistically significant portion of subjects can be efficiently prevented from developing a disease or condition referred to herein in the future. Whether a portion is statistically significant can be determined without further ado by the person skilled in the art using various well known statistic evaluation tools as referred to elsewhere in this specification.

[0082] The compound referred to in accordance with the present invention and combinations thereof, may be administered in a variety of dosage forms which include, but are not limited to, liquid solutions or suspensions, tablets, pills, powders, suppositories, polymeric microcapsules or microvesicles, liposomes, and injectable or infusible solutions. The preferred form depends upon the mode of administration and the therapeutic application.

[0083] The route of administration can include the typical routes including, for example, orally, subcutaneously, transdermally, intradermally, rectally, vaginally, intramuscularly, intravenously, intraarterially, by direct injection to the brain, and parenterally. The pharmaceutical preparation also may be adapted for topical use. In addition, in some circumstances, pulmonary administration may be useful, e.g., pulmonary sprays and other respirable forms.

[0084] In addition to pulmonary sprays, intranasal (IN) delivery (for example by nasal sprays) is a preferred application mode of delivering the compositions of the present invention for neurological/psychiatric conditions. Intranasal delivery is well suited to serve as application mode of proteins and peptides, and is very convenient to use, especially for long-term treatments. Examples for the use of intranasal delivery (nasal sprays) for applying peptides or proteins to the brain can be found in: *(*Lyritis & Trovas, Bone 2002; 30:71S-74S*,* Dhillo & Bloom, Curr Opin Pharmacol 2001; 1:651-655*,* Thorne & Frey, Clin Pharmacokinet 2001; 40:907-946*,* Tirucheral et al., Expert Opin Biol Ther 2001; 1:49-66*,* Jin et al., Ann Neurol 2003 ; 53:405-409*,* Lemere et al., Neurobiol Aging 2002; 23:991-1000*,* Lawrence, Lancet 2002; 359:1674*,* Liu et al., Neurosci Lett 2001; 308:91-94*).* For intranasal application, the compounds described herein can be combined with solvents, detergents and substances that increase penetration of the nasal epithelium or delivery into blood vessels, such as drugs that widen nasal blood vessel, increase perfusion, etcetera.

[0085] The above-described substances according to the invention can be formulated for medical purposes according to standard procedures available in the art, e.g., a pharmaceutically acceptable carrier (or excipient) can be added. A carrier or excipient can be a solid, semisolid or liquid material which can serve as a vehicle or medium tor the active ingredient. The proper form and mode of administration can be selected depending on the particular characteristics of the product selected, the disease, or condition to be treated, the stage of the disease or condition, and other relevant circumstances *(*Remington's Pharmaceutical Sciences, Mack Publishing Co. (1990*)).* The proportion and nature of the pharmaceutically acceptable carrier or excipient are determined by the solubility and chemical properties of the substance selected the chosen route of administration, and standard pharmaceutical practice. Topical formulations may also contain penetration enhancers such as oleic acid, propylene glycol, ethanol, urea, lauric diethanolamide or azone, dimethyl sulphoxide, decylmethyl sulphoxide, or pyrrolidone derivatives. Liposomal delivery systems may also be used.

[0086] Preferably, the subject to be treated in the uses and methods of the present invention is a mammal , more preferably, a guinea pig, dog, cat, rat, mouse, horse, cow, sheep, monkey or chimpanzee, most preferably it is a human.

[0087] A therapeutically effective amount of the compound for treating a neurological condition when the factors are used either singularly or in combination should be used in an amount that results in a neuroprotective and/or neuroregenerative effect. Such an effect can be assessed by the assays described herein. The amount preferably range from about 0,01 to about 10 mg/kg body weight per compound or as a combination and can be determined based on age, race, sex, mode of administration and other factors based on the individual patient. The compound can be administered as a single bolus or repeatedly, e.g. as a daily dose, depending on treated neurological condition and on the route of administration. When the compounds are administered in combination, they may be premixed prior to administration, administered simultaneously, or administered singly in series. In certain embodiments of treating neurological conditions as described herein, higher doses of the compound described herein can be especially useful, for example, at least 10

mg/kg body weight, at least 50 mg/kg body weight, or at least 200 mg/kg body weight may be used. A dose of 10 mg/kg body weight (i.v, bolus) has been shown by the inventors to be an effective amount for the treatment of stroke in a rat model (Example 7), Piperine has been found in rat brain tissue with a maximum concentration of about 4 μg/ml following a i.v. bolus of 50 mg/kg (Sunkara at al., Pharmazie 2001; 56:640-642*).* The person skilled in the art knows approaches to infer a human dosage, from animal models (Boxenbaum & DiLea, J Clin Pharmacol 1995; 35:957-966*).* The oral administration of piperine to human patients in an amount of approximately 5 to 20 mg daily for the purpose of enhanced bioavailability of co-administrated drugs has been already reported *(*US 5616593*;* US 5536506*;* Pattanaik et al., Phytother Res. 2006; 20:683-686*).* At this dosage piperine seems to be well tolerated.

[0088] In the case of chronic neurodegenerative processes, such as ALS and dementia, treatment will more likely consist in one daily or preferably use slow-release formulations.

[0089] In another embodiment, the present invention also provides a device especially suited for slow release and constant long-term application that may be an implanted mini-pump, preferably implanted sub-cutaneously (for example, as described in Edith Mathiowitz; (Cd.), Encyclopedia of Controlled Drug Delivery, John Wiley & Sons 1999; 2:896-920*).* Such pumps are known to be useful in insulin therapy. Examples of such pumps include those manufactured/distributed by Animas, Dana Diabecare, Deltec Cozm, Disetronic Switzerland, Medtronic, and Nipro Amigo as well as those described, for example, in U.S. Pat. Nos. 5,474,552*;* 6,558,345*;* 6,122,536*;* 5,492,534*; and* 6,551,276, the relevant contents of which are incorporated herein by reference.

[0090] In one embodiment, the medicament can further comprise one or more additional factors. "Additional factors" according to the invention are any substances that further support the beneficial effect of the medicament. This support can be either cumulative or synergistic. Suitable additional factors are e.g. factors with neuroprotective effects such as erythropoietin, BDNF, VEGF, CTNF, G-CSF and GM-CSF or inflammation modulating factors. Adding bradykinin or analogous substances to an intravenous application of any preparation will support its delivery to the brain, or spinal cord *(*Emerich et al., Clin Pharmacokinet 2001; 40:105-123*;* Siegal et al., Clin Pharmacokinet 2002; 41:171-186*).* Antiapoptotic agents or agents that facilitate the passage over the blood brain berrier may also be used. A person skilled in the art is familiar with the additional factors that are beneficial for the treatment of the individual illnesses.

[0091] Also preferred are modifications or pharmaceutical formulations of the medicament according to the invention that increase its ability to cross the blood-brain-barrier, or shift its distribution coefficient towards brain tissue.

[0092] In the present application it is demonstrated that the above mentioned compounds trigger the differentiation of neuronal stem cells towards a neuronal phenotype of the cells. The importance in neurogenesis reasons the applicability and usefulness of treatment with the compounds according to the present invention in all facets of neurodegenerative diseases, and all conditions where neurons die. In contrast to acting on endogenous stem cells in the brain for the treatment of neurological conditions, piperine or derivatives thereof can be applied to in vitro manipulations of stem cells, for example differentiation and proliferation.

[0093] Therefore in another embodiment of the invention, the compounds can be used to facilitate culturing of stem cell, such as, for example, neural stem cells. In this method, the compounds can be added to the media and premixed before adding to the cells or can be added into the media in which the cells are being cultured.

[0094] Thus, the present invention relates, furthermore, to a method for the in vitro differentiation of stem cells comprising contacting stem cells with at least on compound as defined above. Preferably, the stem cells used in the method are neuronal stem cells.

[0095] The differentiated stem cells obtainable by the aforementioned method are useful for the preparation of a pharmaceutical composition for treating a neuronal condition as specified elsewhere in this specification.

[0096] Accordingly, in one embodiment of the present invention relates to stimulating the growth and differentiation of neuronal stem cells or precondition neuronal stem cells prior to implantation into a mammal using the aforementioned compounds according to the present invention. A further embodiment of this method is to utilize these neuronal stem cells in methods for treating neurological disease as described herein, preferably in methods which provide a neuroprotective effect when the neuronal stem cells are administered to the individual.

[0097] The stem cells can be administered intravenously or intraarterially. It has been shown, for example, in cerebral ischemia or traumatic brain injury, that bone marrow stromal cells injected i.v. find their way to target areas in the brain *(*Mahmood et al., Neurosurgery 2001 ; 49: 1196-1204*;* Lu et al., J Neurotrauma 2001 ; 18;813-819*;* Lu et al., Cell Transplant 2002; 11:275-281*;* Li et al., Neurology 2002; 59:514-523*).* Stem cells may thus be treated by piperine or derivatives thereof in vitro, and then injected via different routes to patients with any of the diseases described herein.

[0098] Finally, the present invention encompasses a kit comprising the compounds referred to in accordance with the present invention in a suitable formulation for the in vitro differentiation of neuronal stem cells and other cells which are derived from neuronal stem cells.

Brief description of the figures

[0099]

Figure 1:

Piperine significantly stimulates the differentiation of neural stem cells to neurons in a dose dependent manner. The differentiation of neural stem cells to neurons is measured by the relative induction of the beta-III tubulin promoter controlled firefly luciferase activity versus the constitutively expressed Renilla luciterase activity. The effect of piperine in a range from 0,2 μM to 100 μM is shown in comparison to the sham treated cells and to the 1 μM retinoic acid (RA) treated cells. Ratios are given in means with SEM as error bar.

Figure 2a:

Chemical formula of 1-(3-trans-benzo-1,3-dioxol-5-ylacryloyl) cyclohexylamine

Figure 2b:

Chemical formula of 1-(3-trans-benza-1,3-dioxol-5-ylacryloyl) cycloheptylamine

Figure 2c:

Chemical formula of 1-(3-trans-benzo-1,3-dioxol-5-ylacryloyl) cyclopentylamine

Figure 2d:

Chemical formula of 1-(3-trans-benzo-1,3-dioxol-5-ylacryloyl) pyrrolidine

Figure 2e:

1-(3-trans-benzo-1,3-dioxol-5 ylacryloyl) cyclohexylamine significantly stimulates the differentiation of neural stem cells to neurons in a dose dependent manner. Analogously to Fig. 1, the effect of 1-(3-trans benzo-1,3-dioxol-5-ylacryloyl) cyclohexylamine (Fig. 2 a) in a range from 0,2 μM to 100 μM is shown in comparison to the sham treated cells and to the 1 μM retinoic acid (RA) treated cells. Ratios are given in means with SEM as error har.

Figure 2 f:

1-(3-trans-benzo-1,3-dioxol-5-ylacryloyl) cycloheptylamine significantly stimulates the differentiation of neural stem cells to neurons in a dose dependent manner.
Analogously to Fig. 1, the effect of 1-(3-trans-benzo-1,3-dioxol-5-ylacryloyl) cycloheptylamine (Fig. 2 b) in a range from 0,2 μM to 100 μM is shown in comparison to the sham treated cells and to the 1 μM retinoic acid (RA) treated cells. Ratios are given in means with SEM as error bar.

Figure 2 g:

1-(3-trans-benzo-1,3-dioxol-5-ylacryloyl) cyclopentylamine significantly stimulates the differentiation of neural stem cells to neurons in a dose dependent manner.
Analogously to Fig. 1, the effect of 1-(3-trans-benzo-1,3-dioxol-5-ylacryloyl) cyclopentylamine (Fig. 2c) In a range from 0,2 μM to 100 μM is shown in comparison to the sham treated cells and to the 1 μM retinoic acid (RA) treated cells. Ratios are given in means with SEM as error bar.

Figure 2 h:

1-(3-trans-benzo-1,3-dioxol-5-ylacryloyl) pyrrolidine significantly stimulates the differentiation of neural stem cells to neurons in a dose dependent manner.
Analogously to Fig. 1, the effect of 1-(3-trans-benzo-1,3-dioxol-5-ylacryloyl) pyrrolidine (Fig. 2 d) in a range from 0,2 μM to 100 μM is shown in comparison to the sham treated cells and to the 1 μM retinoic acid (RA) treated cells. Ratios are given in means with SEM as error bar.

Figure 3:

Piperine has a significant anti-apoptolic effect on neurons in a dose dependent manner.
After incubation of SH-SY5Y neuronal cells with either staurosporine (0,1 μM) alone or in combination with piperine (100 pM to 1U μM) the subsequent apoptosis of the cells is measured using the Caspase-Glo 3/7 Assay Kit (Promega). Arbitrary luminometric signals of the assay as a measure for the apoptosis are given in means with SEM as error bar. As negative control the luminometric signal of sham treated cells is shown.

Figure 4:

Derivatives of piperine have a significant anti-apoptotic effect on neurons.
After incubation of SH-SYSY neuronal cells with piperine (pip) and derivatives thereof (1-(3-trans-benzo-1,3-dioxol-5-ylacryloyl) cyclohexylamine (A; Fig. 2a), 1-(3-trans-benzo-1,3-dioxel-5-ylacryloyl) cycloheptylamine (B; Fig. 2b), 1-(3-trans-benxo-1,3-dioxol-5-ylatryloyl) cyclopentylamine (C; Fig. 2c), 1-(3-trans-benzo-1,3-dioxol-5-ylacryloyl) pyrrolidine (D; Fig. 2d)) each in a concentration of 1 μM each alone and in co-incubation with staurosporine (SP; 0,2 μM) the subsequent apoptosis of the cells is measured using the Caspase-Glo 3/7 Assay Kit (Promega). The degree of apoptosis is compared with the one nf sham treated cells (negative control) and of staurosporine (SP; 0,2 μM) treated cells (positive control). Arbitrary luminometric signals of the assay as a measure for the apoptosis are given in means with SEM as error bar. As negative control the luminometric signal of sham treated cells is shown. Piperine and derivatives exhibit a significant reduction of the staurosporine induced apoptosis.

Figure 5:

Piperine does not diminish the viability of neuronal cells.
The viability of SH-SY5Y neuronal cells after incubation with piperine (final concentration of 0,1 nM to 100 μM) has been determined. Therefore, the cells have been stably transfected with a constitutively expressing luciferase construct prior to the incubation. After the incubation the viability of the cells has been evaluated by measuring the luciferase activity. Arbitrary luminometric signals of the assay as a measure for the remaining viability are given in means with SEM as error bar. As negative and positive control the luminometric signal of sham and staurosporine (0,1 μM) treated cells is shown, respectively.

Figure 6 a:

The TRPV1 receptor ligand capsaicin does not stimulate the differentiation of neural stem cells to neurons.
Analogously to Fig. 1, the effect of capsaicin in a range from 0,1 nM to 100 μM is shown in comparison to the sham treated cells and to the 1 μM retinoic acid (RA) treated cells. Ratios are given in means with SEM as error bar.

Figure 6b:

The TRPV1 receptor ligand capsaicin exhibit comparably weak antl-apoptotic activity on neurons. After incubation of SH-SYSY neuronal cells with either staurosporine (SP; 0,1 μM) alone or in combination with capsaicin (10 μM) or with piperine (10 μM) the subsequent apoptosis of the cells is measured using the Caspase-Glo 3/7 Assay Kit (Promega). Arbitrary luminometric signals of the assay as a measure for the staurosporine induced apoptosis arc given in means with SFM as error bar. As negative control the luminometric signal of sham treated cells is shown.

Figure 6 c:

The TRPV1 receptor Inhibitor SB366791 does not diminish the stimulating effect of piperine on the differentiation of neural stem cells to neurons.
Analogously to Fig. 1, the effect of 10 μM piperine alone and in combination with the TRPV1 receptor inhibitor SB366791 (10 μM) is shown in comparison to the sham treated cells and to the 1 μM retinoic acid (RA) treated , cells. Ratios are given in means with SEM as error bar.

Figure 6 d:

The TRPV1 receptor inhibitor SB366791 does not diminish the anti-apoptotic effect of piperine on neuronal cells.
After incubation of SH-SY5Y neuronal cells with either staurosporine (SP; 0,1 μM) alone, in combination with

piperine (pip; 10 μM), or with piperine (pip; 10 μM) plus SB366791 (10 nM to 100 nM) the subsequent apoptosls of the cells has been measured analogously to Fig. 3. Arbitrary luminometric signals of the assay as a measure for the apoptosis are given in means with SEM as error bar. As negative control the luminometric signal of sham treated cells is shown.

Figure 7a:

Piperine reduces the infarct volume in the rat MCAO model when applied 30 min after onset of ischemia.
Infarct volumes were determined 24 h after onset of ischemia by TTC-staining. Shown are two exemplary sections of TTC-stained brains of a sham treated and ot a piperine treated animal. The unstained regions marks the infarct volume.

Figure 7b:

Piperine reduces cortical and subcortical infarct volume in the rat MCAO model when applied 30 min after onset of ischemia.
Shown are edema-corrected infarct volumes for sham and piperine treatment for the total infarct, cortical and subcortical areas as determined 24 h after onset of ischemia by TTC-staining and planimetry. Edema-corrected infarct volumes were obtained by deducting the non-infarcted ipsilateral hemisphere volume from the contralateral hemisphere volume. Volumes are given in mm^3 as means with SEM as error bar.

Examples

[0100] The following Examples are provided herein for purposes of illustration only and are not intended to be limiting unless otherwise specified.

Example 1:

In vitro differentiation assay on adult neuronal stem cells with piperine

[0101] Neural stem cells were isolated from the subventricular zone of 4 week-old male Wistar rats as described *(*Maurer et al, Proteome Sci. 2003; 1:4*).* Cells were cultivated in Neurobasal medium (invitrogen) supplemented with B27 (Invitrogen), 2mM L-glutamine, 100units/ml penicillin, 100units/ml streptomycin, 20ng/ml endothelial growth factor (EGF), 20ng/ml fibroblast growth factor-2 (FGH-2), and 2μg/ml heparin. Once a week the neural stem cells were passage and experiments were performed after 4 weeks in vitro. For DNA transfection, the cells were dissociated and plated on poly-L-ornithin/laminin-coated 96-well plates at a density of 50.000 cells/well. Cotransfection with the pGL3-p-BIII-tubulin vector (100ng/well) and the pRL SV40 vector (100ng/well) was carried out according to the FuGene6 Transfection protocol (Roche). The pRL SV40 vector (Promega) served as an internal control vector.

[0102] To amplify the class III B-tubulin gene promoter (fragment -450 - +54), rat genomic DNA was used as a template for PCR *(*Dennis et al., Gene 2002;294:269-277*).* The amplified fragment was inserted into the Mlu I/Xho I site of the pGL3-Basic firefly luciferase reporter vector (Promega) to generate the pGL3-p-BIII-tubulin experimental vector.

[0103] After overnight incubation, the plate was decanted and given in 8-fold replicates fresh medium containing piperine (Sigma) or vehicle. As a positive control for in vitro differentiation, stem cells were treated by adding 1 μM retinoic acid (Sigma) to the medium missing the mitogens.

[0104] After 48hrs, the cells were harvested to prepare the cellular extracts for luciferase assay following the directions of the manufacturer (Promega). As the cells were cotransfected with the firefly and the Renilla luciferase, the Dual Luciferase Reporter Assay System (Promega) was used and the ratio of luminescence signals from the reaction mediated by firefly luciferase to those from the reaction mediated by Renilla luciferase were measured with a luminometer (Berthold Technologies, Mithras LB 940). Variations in the transfection efficiency were normalized using the luminescence signals from the constitutively expressed Renilla luciferase.

[0105] Piperine was used in a range from 0,2 to 100 μM final concentration. The relative induction of the class III β-tubulin gene promoter controlled luciferase signal as a measurement for the neural stem cell differentiation is shown in Fig. 1. The results indicate that piperine significantly stimulates the differentiation of neural stem cells to neurons and therefore acts neuroregenerative.

Example 2:

In vitro differentiation assay on adult neuronal stem cells with derivatives of piperine

[0106] Analogously to Example 1, various derivatives of piperine (1-(3-trans-benzo-1,3-dioxol-5-ylacryloyl) cyclohexylamine (Fig. 2a), 1-(3-trans-benzo-1,3-dioxol-5-ylacryloyl) cycloheptylamine (Fig. 2b), 1-(3-trans-benzo-1,3-dioxol-5-ylacryloyl) cyclopentylamine (Fig. 2c), 1-(3-trans-benzo-1,3-dioxol-5-ylacryloyl) pyrrolidine (Fig. 2d)) (ChemBridge Corporation) have been analysed for their activity to stimulate the differentiation of neural stem cells to neurons. The derivatives were used in a range from 0,2 to 100 μM final concentration. The relative induction of the class III B-tubulin gene promoter controlled luciferase signal as a measurement for the neural stem cell differentiation is shown for these derivatives of piperine in Fig. 2e-h, respectively. The results indicate that all tested derivatives of piperine significantly stimulate the differentiation of neural stem cells to neurons and therefore act neuroregenerative.

Example 3:

In vitro anti-apoptosis assay with piperine

[0107] The neuroprotective effect of compounds can be assayed in vitro by measuring caspase-3 and caspase-7 activities. These members of the cysteine aspartic acid-specific protease (caspase) family play a key effector role in apoptosis in mammalian cells.

[0108] The human neuroblastoma cell-line SH-SYSY (American Type Culture Collection) maintained in DMEM with high glucose supplemented with 20% FBS was used for the apoptosis assay. Alternatively one can analyse the apoptosis ot primary cortical neurons which can be isolated from E18 rats and subsequently cultivated in suitable culture medium. $2,5 \times 10^4$ SH-SYSY cells were seeded in 96-well plates and stimulated 24 h later with piperine or vehicle in the presence of the apoptosis inducing compound staurosporine (0,1 μM, Calbiochem). After 5h incubation at 37 °C, caspase-Glo 3/7 reagent (Promega) containing luciferase and luminogenic substrate was added to the cells. The cleavage of the substrate by caspase-3 and caspase-7 was detected 30 min later using a luminometer (Berthold Technologies, Mithras LB 940). The Luminescence which is proportional to the amount of caspase activity serves as a measure for the apoptosis of the treated neurons. Piperine was tested in a range from 100 pM to 10 μM final concentration. The piperine treated cells showed a significant reduction of the staurosporine induced apoptosis (Fig. 3). The results indicate that piperine exhibits a significant anti-apoptotic effect on neurons and therefore acts neuroprotective.

Example 4:

In vitro anti-apoptosis assay with derivatives of piperine.

[0109] Analogously to Example 3, various derivatives of piperine (1 (3-trans-benzo-1,3-dioxol-5-ylacryloyl) cyclohexylamine (derivative A, Fig. 2a), 1-(3-trans-benzo-1,3-dioxol-5-ylacryloyl) cycloheptylamine (derivative B, 1-ig. 2b), 1-(3-trans-benzo-1,3-dioxol 5 ylacryloyl) cyclopentylamine (derivative C, Fig. 2c), 1-(3-trans-benzo-1,3-dioxol-5-ylacryloyl) pyrrolidine (derivative D, Fig. 2d)) (ChemBridge Corporation) have been analysed for their anti-apoptotic activity. The SH-SY5Y cells have been incubated with these derivatives (1 μM) either alone or in the presence of staurosporine (SP; 0,2 μM). Incubation with staurosporine (SP; 0,2 μM) alone served as positive control and incubation with vehicle served as negative control. The degree of apoptosis relative to the staurosporine control is shown in Fig. 4 (vehicle (sham): 6,0 ± 0,4 %; staurosporine (SP): 100 ± 4,7 %; piperine: 6,4 ± 0,4 %; piperine plus staurosporine: 65,9 ± 3,1 %; derivative A: 6,5 ± 0,4 %; derivative A plus staurosporine: 69,7 ± 3,2 %; derivative B: 5,2 ± 0,4 %; derivative B plus staurosporine: 49,3 ± 1,5 %: derivative C: 7,4 ± 0,3%; derivative C plus staurosporine: 76,6 ± 3,4 %; derivative D: 6,5 ± 0,2 %; derivative D plus staurosporine: 71,7 ± 2,2 %). The cells treated with piperine or derivatives thereof showed a significant reduction of the staurosporine induced apoptosis (Fig. 4). The results indicate that piperine and derivatives thereof exhibit a significant anti-apoptotic effect on neurons and therefore act neuroprotective. Furthermore, neither piperine nor derivatives thereof exhibited an apoptotic effect by themselves.

Example 5:

In vitro viability assay with piperine

[0110] The effect of a compound on the viability of neuronal cells can be assayed in vitro by measuring the survival of neuronal cells transfected with a reporter construct under a constitutive promoter. SH-SYSY neuronal cells stably transfected with a Renilla luciferase construct under the SV40 promoter were seeded in 96-well plates at a density of

2,5 x 10^4 cells per well. Following overnight incubation the cells were stimulated with medium containing piperine or vehicle. Staurosporine (0,1 μM) served as positive control. The Renilla luciferase activity was measured 5 h later using a luminometer (Berthold Technologies, Mithras LB 940). Piperine was tested in a range- from 0,1 nM to 100 μM final concentration. The piperine treated cells did not show any significant effect on the viability of the neuronal cells (Fig. 5). The results indicate that piperine do not reduce the viability of neuronal cells.

Example 6:

[0111] Neuroregenerative and neuroprotective effect of piperine is not mediated by the IRPV1 receptor. Analyses were performed to find out whether the neuroregenerative and/or neuroprotective effect of piperine is mediated via the TRPV1 receptor which is known to be agonized by piperine and other compounds with vanillyl moiety such as capsaicin (Sigma). In an experiment analogous to Example 1 but using capsaicin (0,1 nM to 100 μM final concentration) Instead of piperine no stimulating effect on the differentiation of neuronal stem cells could be detected (Fig. 6 a). Furthermore, capsaicin (10 μM) exhibited only a marginal anti-apoptotic effect (Fig. 6 b) compared to piperine (10 μM) using the assay as described in Example 3.

[0112] Moreover, the compound SB366791 (Sigma) which is a specific inhibitor of the TRPV1 receptor did not result in any inhibition of the neurodegenerative or neuroprotective effect of piperine when co-incubated in the assays of Examples 1 and 3, respectively (Fig. 6c and Fig. 6d, respectively). Neither the TRPV1 receptor agonist capsaicin showed a neuroprotective and/or neuroregenerative effect comparable to piperine nor the TRPV1 receptor Inhibitor SB366791 diminished the neuroprotective and/or neuroregenerative effect of piperine. Therefore, the inventors assumed that this effect of piperine is not mediated via the TRPV1 receptor.

Example 7:

Piperine is neuroprotective in an animal model for stroke '

[0113] As piperine showed distinct anti-apoptotic and neurogenic properties on primary neurons in culture (Examples 1 and 3), and passes the intact blood-brain-barrier, the inventors sougnt to determine its neuroprotective activity in vivo. The inventors chose the rat MCAO (middle cerebral occlusion; filament model) model for stroke, where a broad variety of different neuron types is damaged by ischemia/hypoxia.

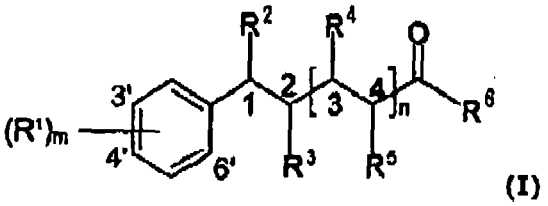
[0114] Male Wistar rats received inhalation anesthesia with 70% N_2O, 30% O_2, and 1% halothane. The right femoral vein was cannulated and used for drug delivery. During the experiment core body temperature was monitored and maintained at 37 °C. Middle cerebral artery occlusion (MCAO) was induced with a silicon-coated (Provil Novo, Heraus Kulzer) 4-0 nylon filament (Ethicon) that was introduced into the common carotid artery and advanced into the internal carotid artery. Successful MCA occlusion was verified by Laser-Doppler flowmetry (Perimed 4000) with a probe positioned over the temporal cortical MCA territory, After 90 min MCAO, the filament was withdrawn to allow for reperfusion. 30 minutes after onset of occlusion animals received 10 mg/kg piperine (Sigma-Aldrich) or vehicle i.v. over 20 min at a rate of 2 μl/min. Piperine was dissolved at 60°C in 100% Solutol-HS15 (BASF), and diluted with aqua dest to a final concentration of 20% Solutol-HS15, resulting in a final concentration of 5 mg Piperine/ml final solution. The solution was kept at room temperature in the dark until needed. Infarct volumes were determined 24 h after induction of ischemia by TTC staining. 2 mm sections were cut using a brain matrix (Harvard Apparatus, Inc.), and stained with 2,3,5-Triphenyl tetrazolium chloride (TTC, Sigma-Aldrich) for 10 min at 37°C. Fig. 7 a shows exemplary TTC stained brain sections after sham treatment and after piperine treatment. Stained sections were scanned on both sides using a colour scanner, and infarct areas determined using Image] (http://rsb.into.nlh.gov/lj). "Direct" infarct volume was obtained by Integrating measured infarcted areas: edema-corrected infarct volume was obtained by deducting the non-infarcted volume of the infarcted hemisphere from the contralateral hemisphere. Animals with any signs of subarachnold hemorrhage (barrel-rolling, or blood in the ventricles or subarachnoid space), any cortical damage from drill holes, and no or minimal infarcts on TTC-staining were excluded from the analysis before unblinding. All experiments were done in a fully randomized and blinded fashion.

[0115] Animals treated with piperine demonstrated a significant reduction of total edema-corrected infarct size (vehicle: 210 ± 20 mm^3, n=37; piperine: 151 ± 17 mm^3; n=30; p=0.031). Particularly strong protection was observed in the cortex (vehicle: 124 ± 15 mm^3; piperine: 73 ± 14 mm^3; p=0.018), whereas the infarct reduction in subcortical areas did not reach statistical significance (vehicle: 86 ± 7 mm^3; piperine; 78 ⊥ 6 mm^3). Volumes are given in mean plus/minus standard error of the mean (SEM) whereas n represents the number of replicates and p represents the p-value result of a Student's ttest. The results are shown in Fig. 7 b.

[0116] As a conclusion of this example piperine acts in a neuroprotective manner in vivo in an animal stroke model. The treatment appeared to be also well tolerated by the animals.

Claims

1. Use of a compound with the general formula (I)



(I)

wherein

n=0, 1, or 2, provided that

when n=0, R^2 and R^3 represent hydrogen atoms or together represent a carbon tn carbon double bond, either In E or in Z geometric configuration;
when n=1, or 2, R^2 and R^3 represent hydrogen atoms or together represent a carbon to carbon double bond, either in E or in Z geometric configuration, and R^4 and R^5 represent hydrogen atoms or together represent a carbon to carbon double bond, either in E or in Z geometric configuration;

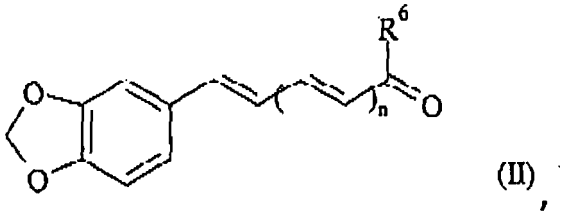
m=0, 1, 2, or 3, provided that

when m=1, R^1 represents an alkoxy group having from 1 to 3 carbon atoms, or a hydroxy group,
when m=2, each R^1 independently represents an alkoxy group having from 1 to 3 carbon atoms or the two R^1 together represent a 3',4'-methylenedioxy group;
when m=3, two R^1 together represent a 3',4'-methylenedioxy group and the other R^1 represents an alkoxy group having from 1 to 3 carbon atoms, or a hydroxy group;

R^6 represents a pyrrolidino, piperidino, 1-methylpiperidino, morpholino, N-monoalkylamino group of 4 to 6 carbon atoms, N-monocycloalkylamino group of 4 to 7 carbon atoms, 3',4'-methylenedioxy-substituted benzylamino group, 2-phenethylamino group, or an alkoxy group having from 1 to 6 carbon atoms

for the preparation of a pharmaceutical composition for treating and/or preventing a neuronal condition.

2. The use of claim 1, wherein the compound of the present invention has the general formula (II)



(II),

wherein

n is 0 or 1 and R^6 has the meaning as indicated in claim 1.

3. The use of claim 1 or 2, wherein R^6 represents a pyrrolidino, piperidino, 4-methylpiperidino, morpholino, N-monoalkylamino group of 4 to 6 carbon atoms, N-monocycloaikylamino group of 4 to 7 carbon atoms, 3',4'-methylenedioxy-substituted benzylamino group, or 2-phenethylamino group.

4. The use of any one of claims 1 to 3, wherein the compound is selected from the group consisting of piperine, Trichostachine, Piperlonguminine, 5-E,E-piperinoyl methylamine, 5-E,E-piperinoyl ethylamine, 5-E,E-piperinoyl iso-

propylamine, 5-E,E-piperinoyl cyclohexylamine, 5-E,E-piperinoyl butylamine, Despiperidyl-methoxypiperidine, 5-E, E-piperinoyl morpholine, 5-E,E-piperinoyl hexylamine, 5-E,E-piperinoyl piperinoylamine, 5-E,E-piperinic acid ethyl ester, 5-E,E-piperinic acid isopropyl ester, 5-E,E-piperinic acid propyl ester, 5-E,E-piperinic acid butyl ester, Antiepilepsirine, 1-(3-trans-benzo-1,3-dioxol-5-ylacryloyl) cyclohexylamine, 1-(3-trans-benzo-1,3-dioxol-5-ylacryloyl) cycloheptylamine, 1-(3-trans-benzo 1,3-dioxol-5-ylacryloyl) cyclopenylamine, 1-(3-trans-benzo-1,3-dioxol-5-ylacryloyl) pyrrolidine, 1-(3-trans-benzo-1,3-dioxol-5-ylacryloyl) morpholine, 3-trans-benzo-1,3-dioxol-5-ylacrylic acid methyl ester, Piperettine, Coumaperine, 4'-Methoxyiso-coumaperine, Wisanine, 1-(4-methoxy-cinnamoyl)piperidine, 1-(3-methoxy-cinnamoyl)piperidine, 1-(2-methoxy-cinnamoyl)piperidine, 1-cinnamoyl-piperidine, 1-(3,4-dimethoxy-cinnamoyl)piperidine, 3-benzo-1,3-dioxol-5-ylpropionic acid piperidide, 1,2,3,4-Tetrahydropiperine, Piperanine, Chavicine, isopiperine, and Isochavicine.

5. The use of any one of claims 1 to 4, wherein the compound is piperine.

6. The use of any one of claims 1 to 5, wherein the neurological condition is at least one condition selected from the group consisting of cerebral ischemia, amyotrophic lateral sclerosis, glaucoma, Alzheimer's disease, neurodegenerative trinucleotide repeat disorders, neurodegenerative lysosomal storage diseases, multiple sclerosis, spinal cord injury, spinal cord trauma, dementia, schizophrenia, and peripheral neuropathy.

7. The use of any one of claims 1 to 6, wherein the neurological condition is a neurological disease with a pathophysiological mechanism involving ischemia and/or hypoxia selected from the group consisting of stroke, traumatic brain injury, cerebral ischemia due to cardiocirculatory arrest, glaucoma, spinal cord injury, and spinal cord trauma.

8. Use of a compound as defined in any one of claims 1 to 7 for the preparation of a pharmaceutical composition for enhancing learning and memory.

9. A method of treating a patient with a neurological condition comprising administering in a therapeutically effective amount a compound as defined in any one of claims 1 to 5 to a patient suffering from the said neurological condition.

10. The method of claim 9, wherein said neurological condition is at least one condition selected from the group consisting of cerebral ischemia, amyotrophic lateral sclerosis, glaucoma, Alzheimer's disease, neurodegenerative trinucleotide repeat disorders, neurodegenerative lysosomal storage diseases, multiple sclerosis, spinal cord injury, spinal cord trauma, dementia, schizophrenia, and peripheral neuropathy.

11. The method of claim 10, wherein said neurological condition is a neurological disease with a pathophysiological mechanism involving ischemia and/or hypoxia selected from the group consisting of stroke, traumatic brain injury, cerebral ischemia due to cardiocirculatory arrest, glaucoma, spinal cord injury, and spinal cord trauma

12. A method for the in vitro differentiation of stem cells comprising contacting stem cells with at least one compound as defined in any one of claims 1 to 5.

13. The method of claim 12, wherein the stem cells are neuronal stem cells.

14. Use of differentiated stem cells obtainable by the method of claim 12 or 13 for the preparation of a pharmaceutical composition for treating a neuronal condition.

15. Kit for the in vitro differentiation of neuronal stem cells comprising at least one compound as defined in any one of claims 1 to 5.

Figures

Fig. 1

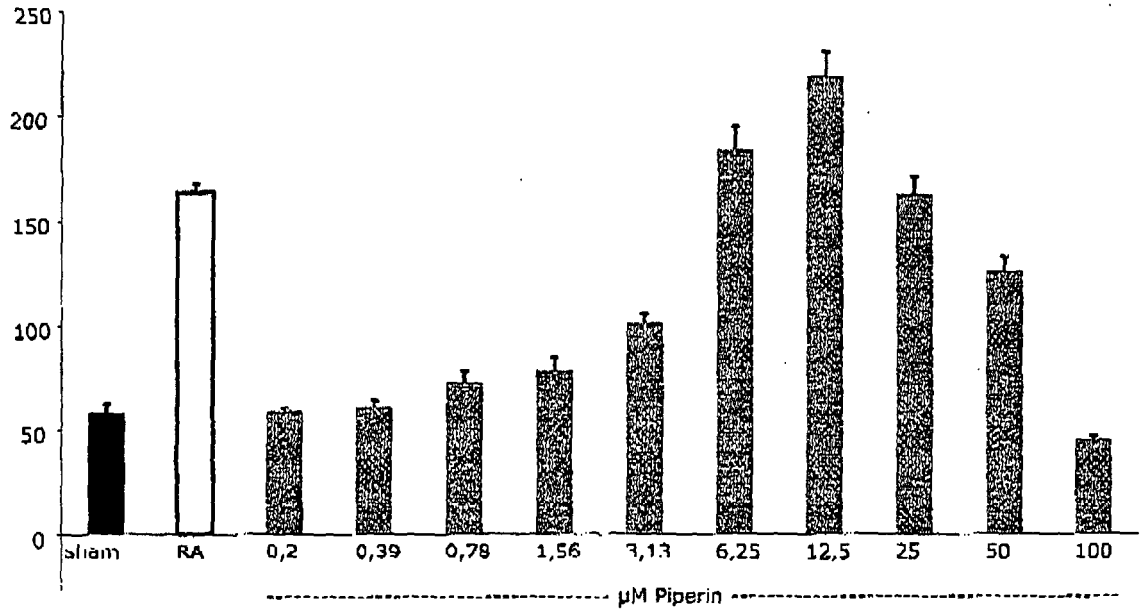


Fig. 2a

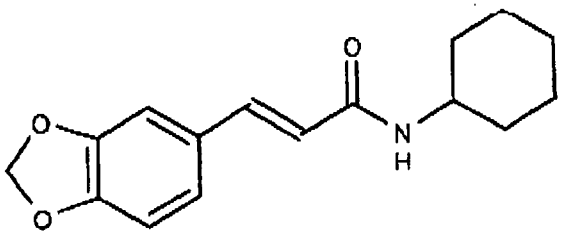


Fig. 2b

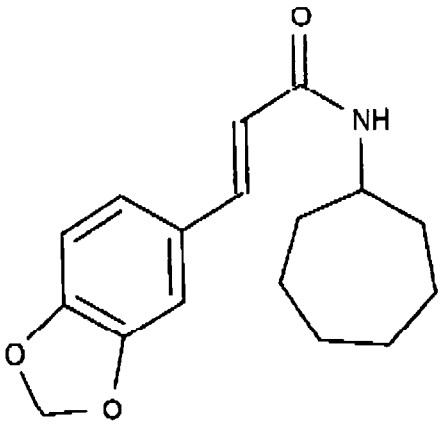


Fig. 2c

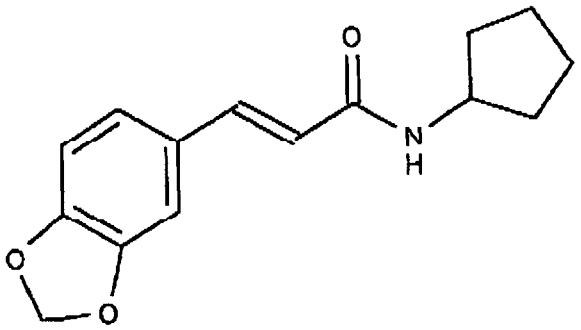


Fig. 2d

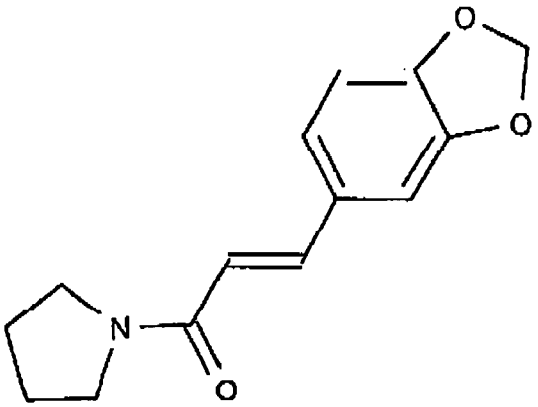


Fig. 2e

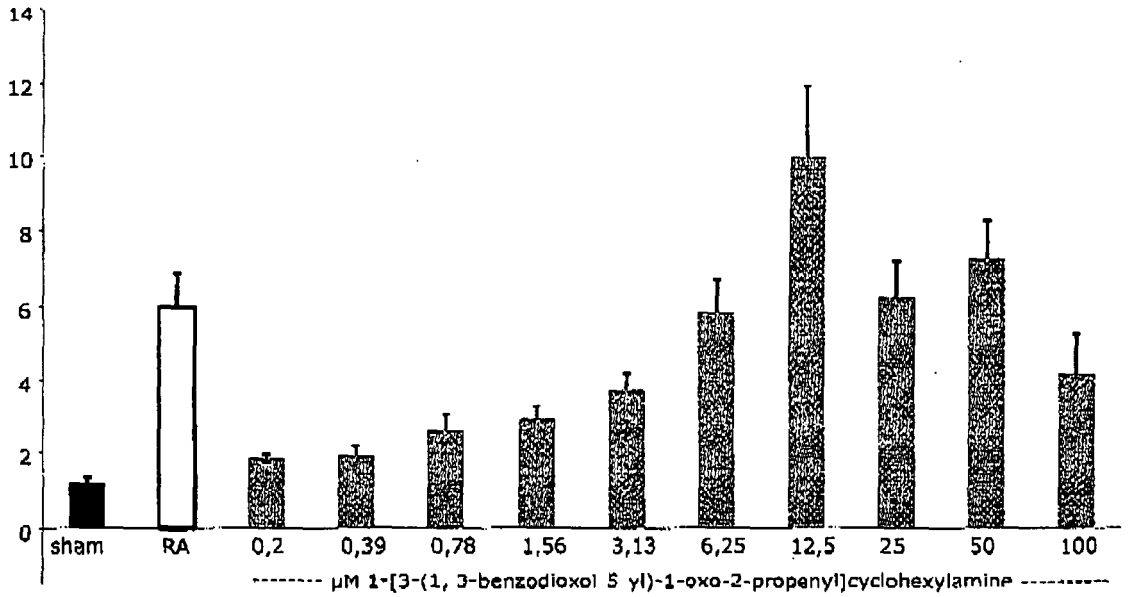

14
12
10
8
6
4
2
0
sham
RA
0,2
0,39
0,78
1,56
3,13
6,25
12,5
25
50
100
-------- µM 1-[3-(1, 3-benzodioxol 5 yl)-1-oxo-2-propenyl]cyclohexylamine ----------

Fig. 2f

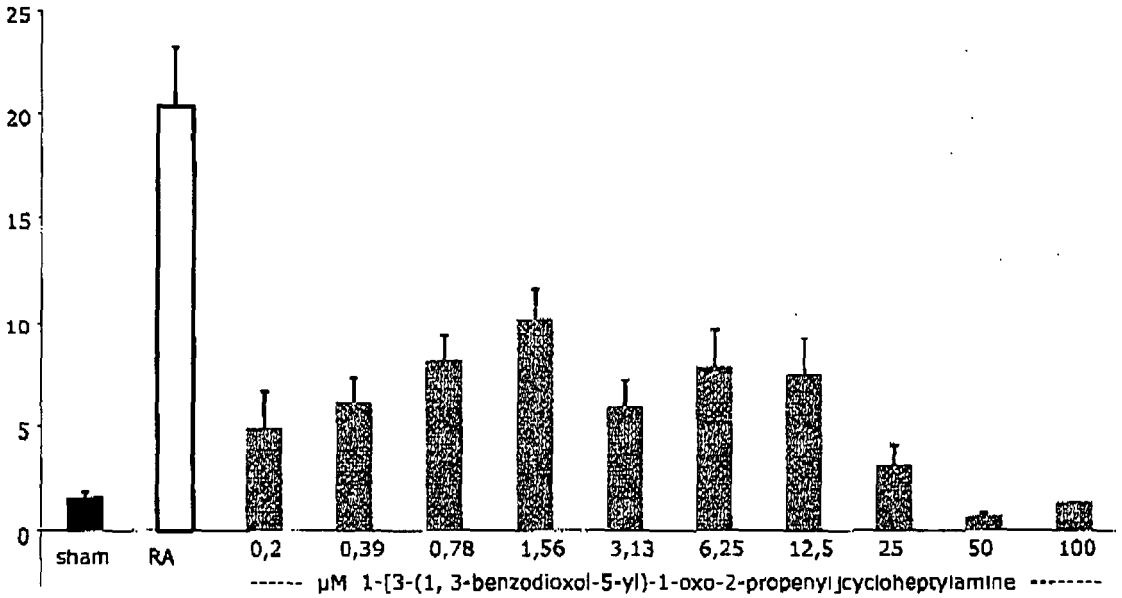

25
20
15
10
5
0
sham
RA
0,2
0,39
0,78
1,56
3,13
6,25
12,5
25
50
100
------ µM 1-[3-(1, 3-benzodioxol-5-yl)-1-oxo-2-propenyl]cycloheptylamine --------

Fig. 2g

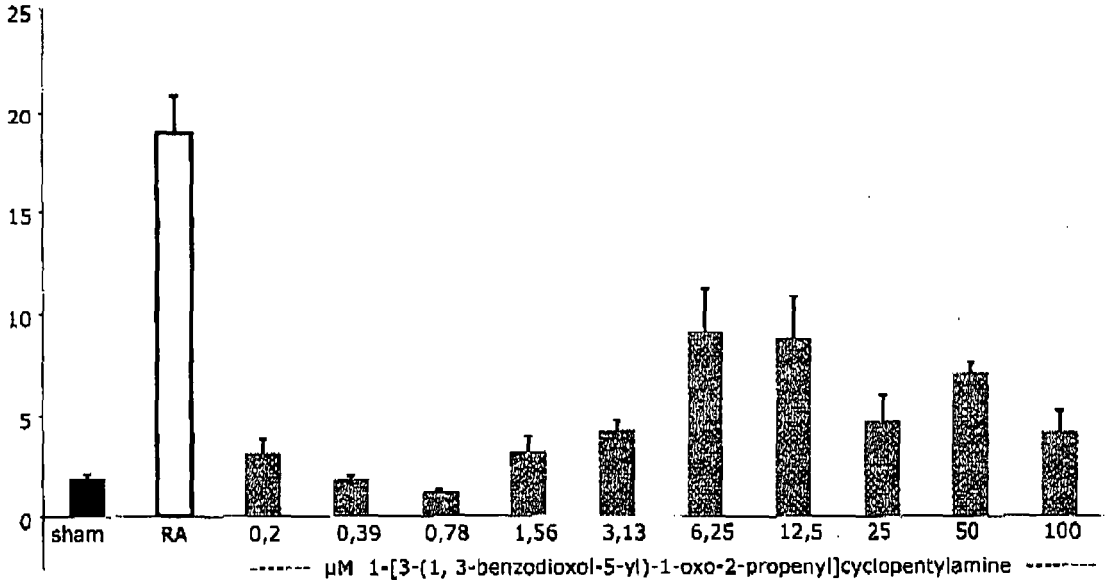

25
20
15
10
5
0
sham
RA
0,2
0,39
0,78
1,56
3,13
6,25
12,5
25
50
100
------- µM 1-[3-(1, 3-benzodioxol-5-yl)-1-oxo-2-propenyl]cyclopentylamine --------

Fig. 2h

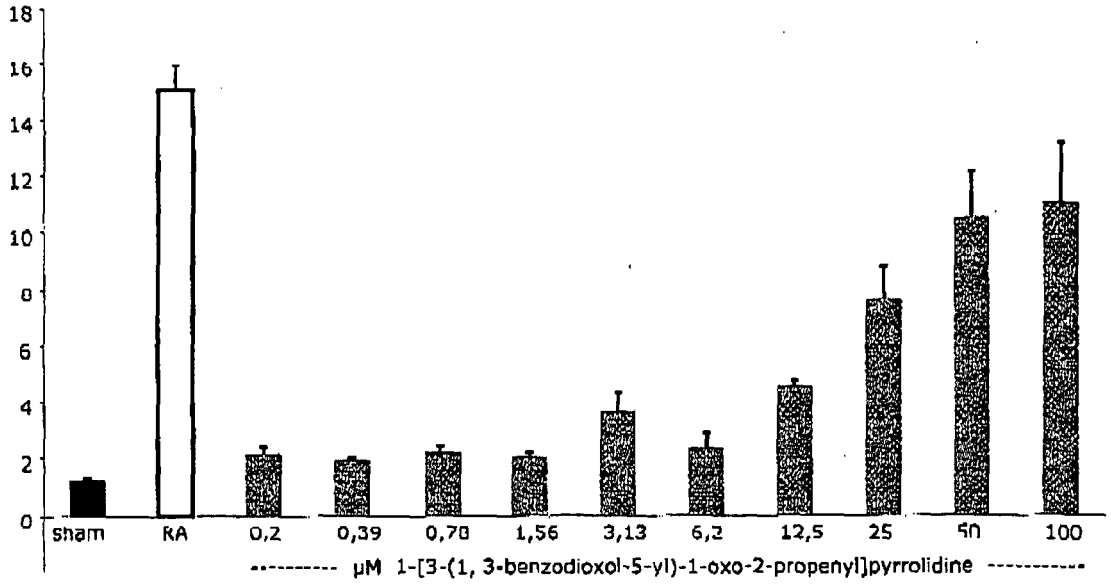

18
16
14
12
10
8
6
4
2
0
sham
RA
0,2
0,39
0,78
1,56
3,13
6,2
12,5
25
50
100
---------- µM 1-[3-(1, 3-benzodioxol-5-yl)-1-oxo-2-propenyl]pyrrolidine ----------

Fig. 3:

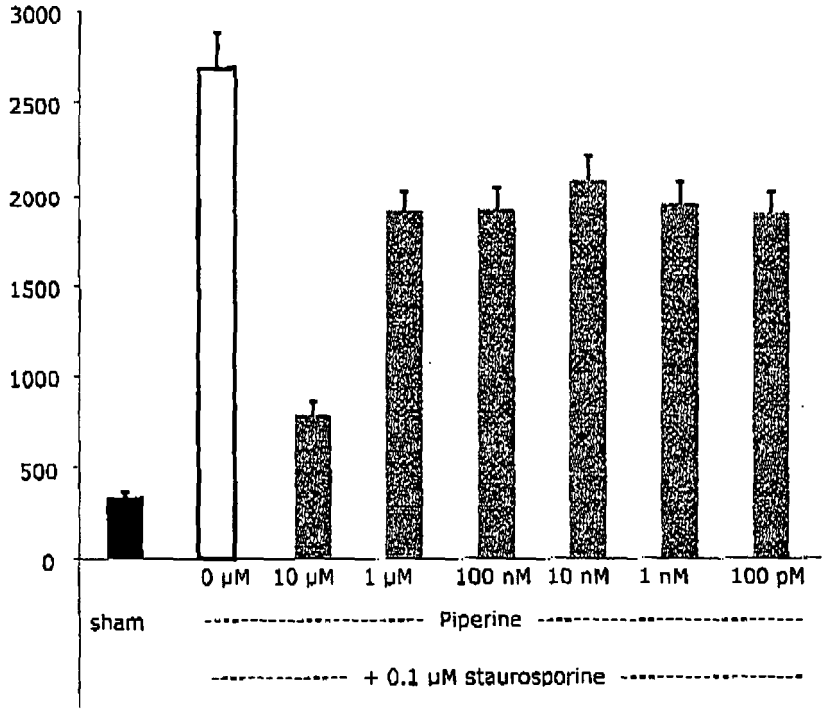

3000
2500
2000
1500
1000
500
0
0 µM
10 µM
1 µM
100 nM
10 nM
1 nM
100 pM
sham
Piperine
+ 0.1 µM staurosporine

Fig. 4:

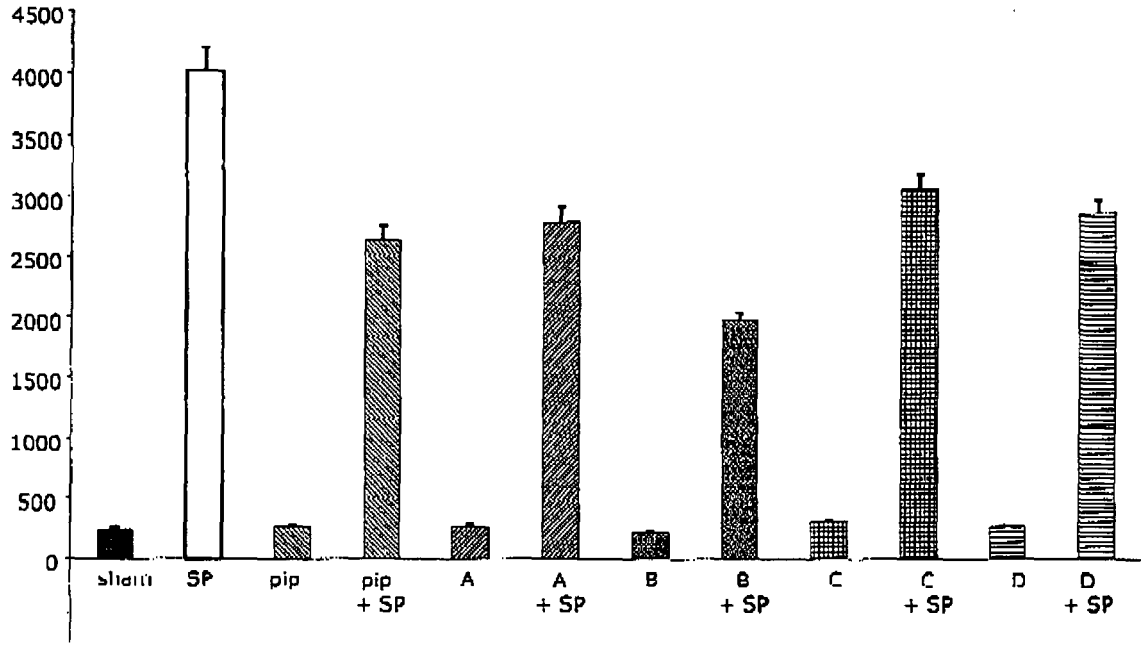

4500
4000
3500
3000
2500
2000
1500
1000
500
0
sham
SP
pip
pip + SP
A
A + SP
B
B + SP
C
C + SP
D
D + SP

Fig. 5:

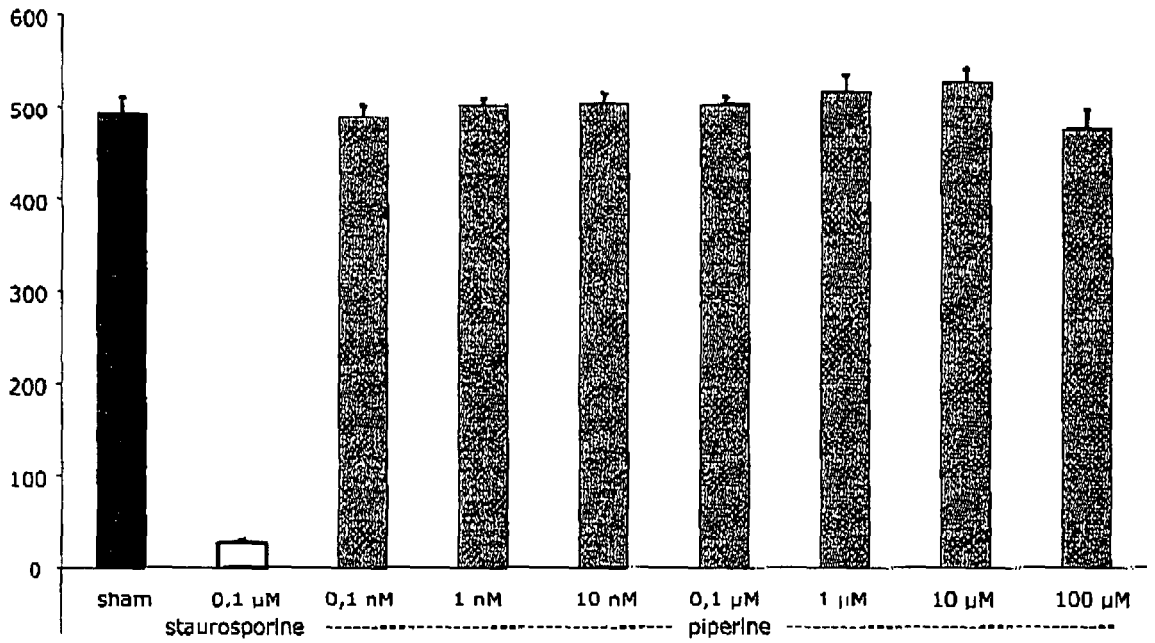

600
500
400
300
200
100
0
sham
0.1 µM staurosporine
0,1 nM
1 nM
10 nM
0,1 µM piperine
1 µM
10 µM
100 µM

Fig. 6 a:

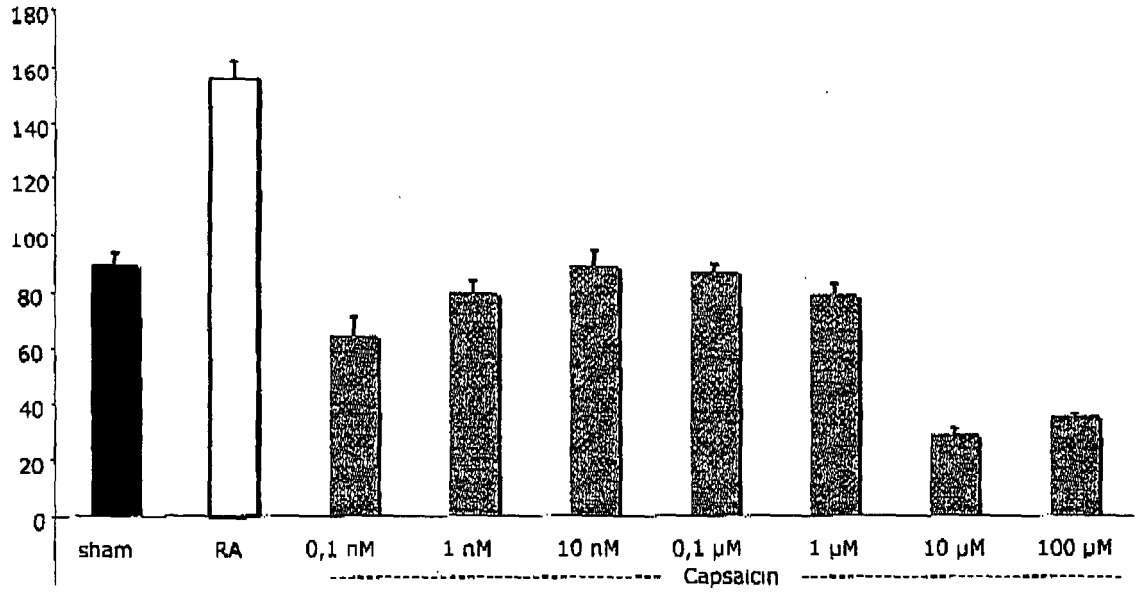

180
160
140
120
100
80
60
40
20
0
sham
RA
0,1 nM
1 nM
10 nM
0,1 µM
1 µM
10 µM
100 µM
Capsaicin

Fig. 6 b:

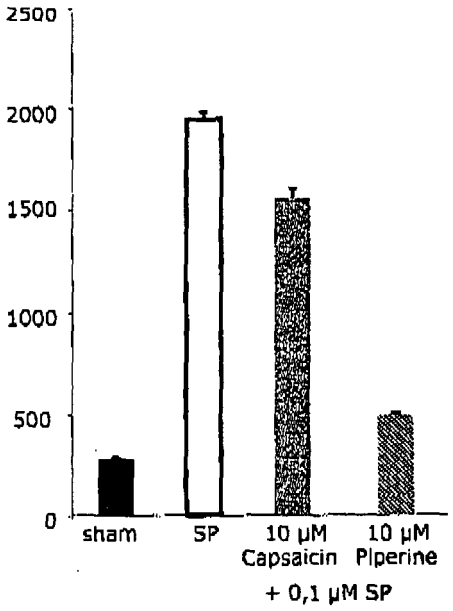

2500
2000
1500
1000
500
0
sham
SP
10 µM Capsaicin + 0,1 µM SP
10 µM Piperine

Fig. 6 c:

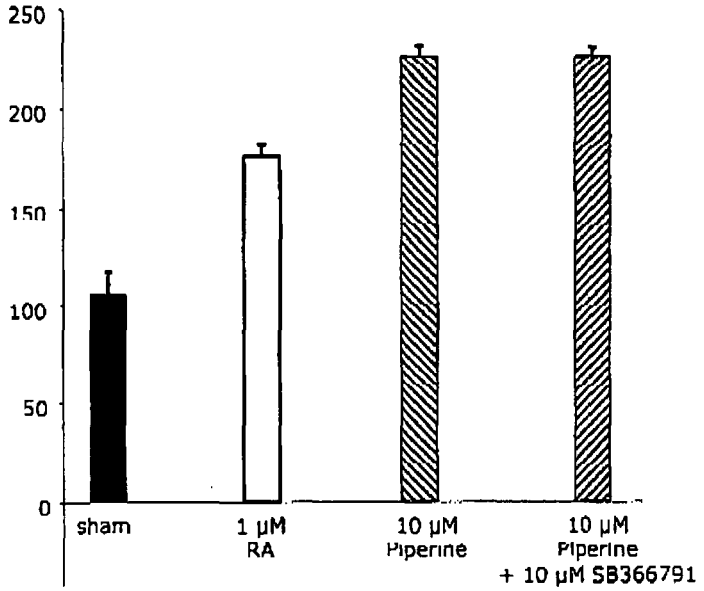

250
200
150
100
50
0
sham
1 µM RA
10 µM Piperine
10 µM Piperine + 10 µM SB366791

Fig. 6 d:

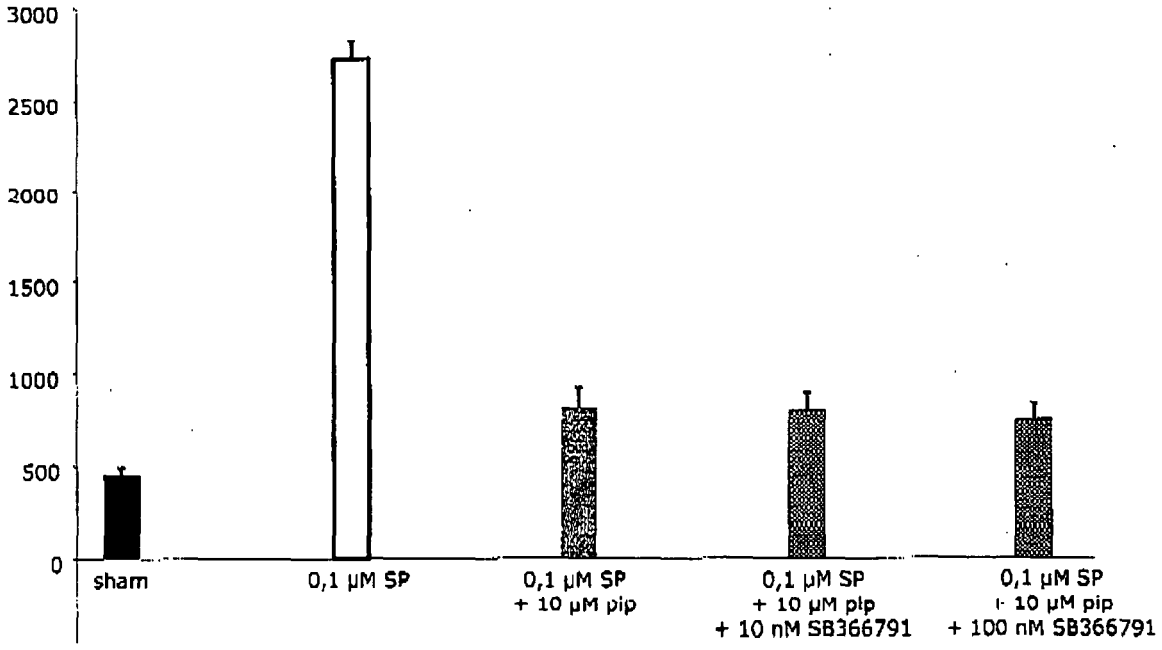

3000
2500
2000
1500
1000
500
0
sham
0,1 µM SP
0,1 µM SP + 10 µM pip
0,1 µM SP + 10 µM pip + 10 nM SB366791
0,1 µM SP + 10 µM pip + 100 nM SB366791

Fig. 7 a:

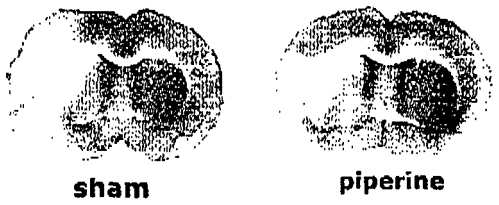
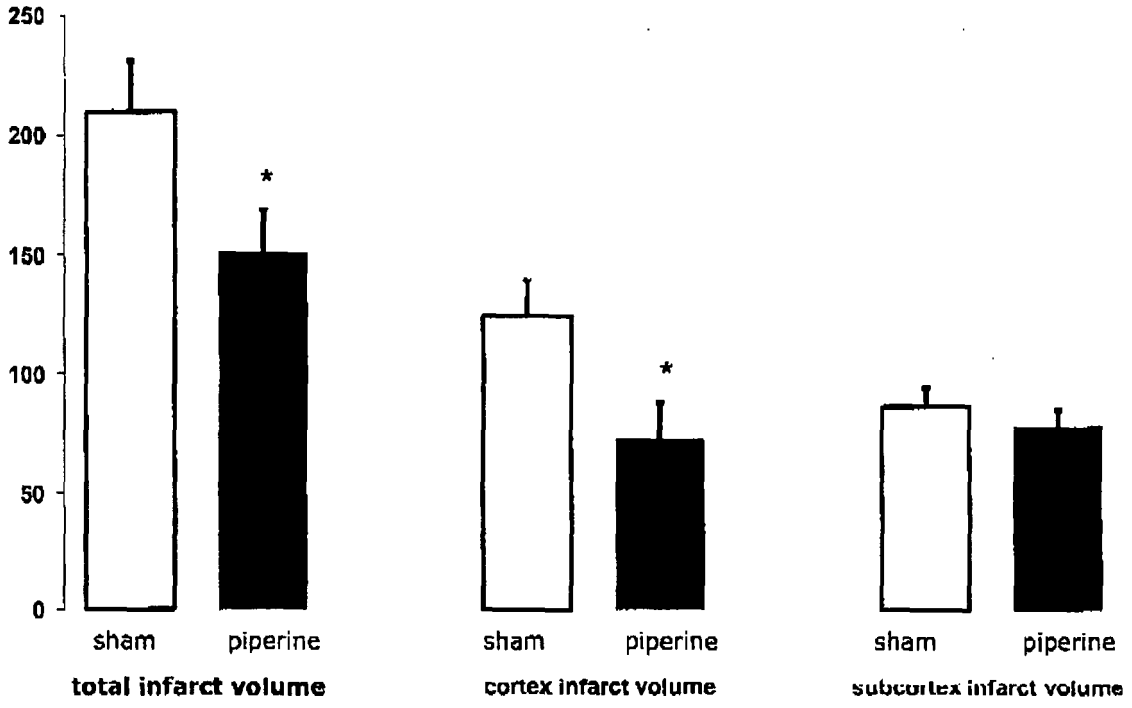

sham
piperine

Fig. 7 b:


250
200
150
100
50
0
*
*
sham
piperine
sham
piperine
sham
piperine
total infarct volume
cortex infarct volume
subcortex infarct volume

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

which under Rule 63 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 07 01 2964

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X	WO 2007/048004 A (CORNELL RES FOUNDATION INC [US]; RATAN RAJIV R [US]; SIDDIQ AMBREENA []) 26 April 2007 (2007-04-26) * page 1, paragraph 6 - page 4, paragraph 1 * * page 5, paragraph 3 - page 10, paragraph 3 * -----	1-15	INV. A61K31/343 A61K31/4015 A61K31/4025 A61K31/45 A61K31/4525 A61K31/5375 A61K31/5377
X	US 2004/001896 A1 (KUPPAM CHANDRASEKHARA RAO [IN]) 1 January 2004 (2004-01-01) * page 1, paragraph 4 * * page 3, paragraphs 33,34,38,40,42 * -----	1-11	A61K36/67 A61P25/00 A61P25/02 A61P25/18 A61P25/28
X	EP 1 281 324 A (DAICHO KIKAKU INC COMPANY [JP]) 5 February 2003 (2003-02-05) * page 2, paragraph 1 * * page 3, paragraphs 17-19,22 * * page 17, paragraph 89 * -----	1-11	A61P27/06
	-/--		

TECHNICAL FIELDS SEARCHED (IPC)

A61K
A61P

INCOMPLETE SEARCH

The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

see sheet C

Place of search	Date of completion of the search	Examiner
Munich	28 November 2007	HOUYVET, C

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04E07)

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 07 01 2964

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (IPC)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X	JOHRI R K ET AL: "AN AYURVEDIC FORMULATION 'TRIKATU' AND ITS CONSTITUENTS" JOURNAL OF ETHNOPHARMACOLOGY, ELSEVIER SCIENTIFIC PUBLISHERS LTD, IE, vol. 2, no. 37, September 1992 (1992-09), pages 85-91, XP002077892 ISSN: 0378-8741 * the whole document *	1-7,9-15	
X	LI ET AL: "Antidepressant like effects of piperine in chronic mild stress treated mice and its possible mechanisms" LIFE SCIENCES, PERGAMON PRESS, OXFORD, GB, vol. 80, no. 15, 12 March 2007 (2007-03-12), pages 1373-1381, XP005923320 ISSN: 0024-3205 * page 1374, column 1, paragraph 2 * * page 1378 - page 1380 *	1-5,9, 12-15	TECHNICAL FIELDS SEARCHED (IPC)
D,X	LIU G Q ET AL: "Stimulation of serotonin synthesis in rat brain after antiepilepsirine, an antiepileptic piperine derivative." BIOCHEMICAL PHARMACOLOGY 1 DEC 1984, vol. 33, no. 23, 1 December 1984 (1984-12-01), pages 3883-3886, XP002460230 ISSN: 0006-2952 * abstract * * page 3883, column 2 * * page 3886, column 1, paragraph 2 *	1-5,9, 12-15	
X	US 2003/236300 A1 (CAPLAN MICHAEL J [US] ET AL) 25 December 2003 (2003-12-25) * page 24, paragraphs 240,241 * * claims 1,6,12 *	1-6,9,10	

-/--

EPO FORM 1503 03.82 (P04C10) 2

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 07 01 2964

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (IPC)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X	AMERICAN FORMULARY INC.: "Co.Q.Care"[Online] 26 June 2007 (2007-06-26), XP002460231 Retrieved from the Internet: URL:http://web.archive.org/web/20070626074546/http://www.americanformulary.com/coqcare.htm> [retrieved on 2007-11-23] * the whole document *	1-6,8-10	
D,X	PEI Y Q: "A review of pharmacology and clinical use of piperine and its derivatives." EPILEPSIA APR 1983, vol. 24, no. 2, April 1983 (1983-04), pages 177-182, XP009092400 ISSN: 0013-9580 * abstract * * page 178, column 1, paragraphs 1,2 *	1-5,9	TECHNICAL FIELDS SEARCHED (IPC)
X	DATABASE WPI Week 200436 Derwent Publications Ltd., London, GB; AN 2004-386065 XP002460233 & KR 2004 003 668 A (TG BIOTECH INC) 13 January 2004 (2004-01-13) * abstract *	1-5,9	
X	DATABASE WPI Week 200436 Derwent Publications Ltd., London, GB; AN 2004-386328 XP002460234 & KR 2004 008 014 A (TG BIOTECH CO LTD) 28 January 2004 (2004-01-28) * abstract *	1-4,9	

-/--

EPO FORM 1503 03.82 (P04C10)

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 07 01 2964

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (IPC)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X	EP 0 709 098 A (CADILA LAB LTD [IN]) 1 May 1996 (1996-05-01) * page 2, lines 3-6 * * page 2, line 55 - page 3, line 30 *	1-5,9	
X	PARMAR V S ET AL: "Phytochemistry of the genus Piper" PHYTOCHEMISTRY, PERGAMON PRESS, GB, vol. 46, no. 4, October 1997 (1997-10), pages 597-673, XP004293460 ISSN: 0031-9422 * page 598, paragraphs 3,4; table 1 * * pages 605-607 *	1-5,9	
D,X	WO 00/02544 A (BTG INT LTD [GB]; RAMAN AMALA [GB]; LIN ZHIXIU [GB]) 20 January 2000 (2000-01-20) * page 1, lines 2-4 * * page 2, line 23 - page 5, line 2 *	12	TECHNICAL FIELDS SEARCHED (IPC)
Y	EP 0 192 431 A (KYOWA HAKKO KOGYO KK [JP]; IHARA CHEMICAL IND CO [JP]) 27 August 1986 (1986-08-27) * page 1, lines 10,11 * * page 2, lines 14-30 * * page 3, lines 3-7 *	1-15	
Y	SINGH, R.P. ET AL.: "Free Radicals and Oxidative Stress in Neurodegenerative Diseases : Relevance of Dietary Antioxidants" JOURNAL, INDIAN ACADEMY OF CLINICAL MEDICINE, vol. 5, no. 3, 2004, pages 218-225, XP002460232 * abstract * * table III * * page 223, column 2, paragraph 1 - page 224, column 2, paragraph 1 *	1-15	

EPO FORM 1503 03.82 (P04C10) 2

European Patent Office

INCOMPLETE SEARCH SHEET C

Application Number
EP 07 01 2964

Although claims 9-11 are directed to a method of treatment of the human/animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the compound.

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 07 01 2964

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

28-11-2007

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2007048004 A	26-04-2007	NONE	
US 2004001896 A1	01-01-2004	AU 2003231061 A1	19-01-2004
		EP 1519738 A1	06-04-2005
		JP 2006508902 T	16-03-2006
		WO 2004002507 A1	08-01-2004
EP 1281324 A	05-02-2003	CA 2396049 A1	31-01-2003
		CN 1406516 A	02-04-2003
		JP 2003245054 A	02-09-2003
		US 2003157199 A1	21-08-2003
US 2003236300 A1	25-12-2003	NONE	
KR 2004003668 A		NONE	
KR 2004008014 A		NONE	
EP 0709098 A	01-05-1996	AU 686656 B2	12-02-1998
		AU 7599294 A	18-05-1995
		CA 2134610 A1	30-04-1995
		CN 1121412 A	01-05-1996
		IN 176897 A1	28-09-1996
		US 5616593 A	01-04-1997
		ZA 9408348 A	14-06-1995
WO 0002544 A	20-01-2000	AT 249220 T	15-09-2003
		AU 757053 B2	30-01-2003
		AU 4920999 A	01-02-2000
		CA 2337205 A1	20-01-2000
		CN 1330545 A	09-01-2002
		DE 69911210 D1	16-10-2003
		DE 69911210 T2	01-07-2004
		EP 1094813 A2	02-05-2001
		ES 2207252 T3	16-05-2004
		HK 1033264 A1	16-07-2004
		JP 2002520275 T	09-07-2002
		US 6346539 B1	12-02-2002
		ZA 200004260 A	23-05-2001
EP 0192431 A	27-08-1986	DE 3687283 D1	28-01-1993
		DE 3687283 T2	22-04-1993
		JP 1949243 C	10-07-1995
		JP 6076377 B	28-09-1994
		JP 62005954 A	12-01-1987
		US 4962107 A	09-10-1990

EPO FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 07 01 2964

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

28-11-2007

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0192431 A		US 4824842 A	25-04-1989

EPO FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- US 5744161 A **[0041] [0056]**
- US 6054585 A **[0041] [0056]**
- US 6365601 B **[0041] [0056]**
- US 5616593 A **[0047] [0087]**
- US 5972382 A **[0047]**
- US 6017932 A **[0047]**
- EP 0650728 A **[0047]**
- EP 1494749 A **[0047]**
- EP 1094813 A **[0050] [0055] [0056]**
- US 6346539 B **[0056]**
- US 5536506 A **[0087]**
- US 5474552 A **[0089]**
- US 6558345 A **[0089]**
- US 6122536 A **[0089]**
- US 5492534 A **[0089]**
- US 6551276 A **[0089]**

Non-patent literature cited in the description

- **CHEN et al.** *Neuroscience,* 2002, vol. 111, 761-773 **[0004] [0004]**
- **KAAS.** *Annu Rev Neurosci.,* 1991, vol. 14, 137-167 **[0004] [0004]**
- **JONES.** *Cereb Cortex,* 1993, vol. 3, 361-372 **[0004]**
- **HESS ; DONOGHUE.** *Neurophysiol,* 1994, vol. 71, 2543-2547 **[0004]**
- **FISHER et al.** *Stroke,* 2006, vol. 37, 1129-1136 **[0004]**
- **LEKER ; SHOHAMI.** *Brain Res. Rev.,* 2002, vol. 39, 55-73 **[0005] [0005]**
- **ZHENG et al.** *Circulation,* 2001, vol. 104, 2158-2163 **[0007]**
- **BOTTIGER et al.** *Heart,* 1999, vol. 82, 674-679 **[0007]**
- **SAFAR.** *Circulation,* 1986, vol. 74, UV138-153 **[0007] [0007] [0007]**
- **HOSSMANN.** *Resuscitation,* 1993, vol. 26, 225-235 **[0007]**
- **SAFAR et al.** *Crit Care Med,* 2002, vol. 30, 140-144 **[0007] [0007]**
- **ADAMS et al.** Principles of Neurology. 1090 1095 **[0008]**
- **CLEVELAND ; ROTHSTEIN.** *Nat Rev Neurosci.,* 2001, vol. 2, 806-819 **[0008]**
- **ISHIGAKI et al.** *J Neurochem.,* 2002, vol. 82, 576 584 **[0008]**
- **LI et al.** *Science,* 2000, vol. 288, 335-339 **[0008]**
- **LEVIN.** *Ophthalmol Clin North Am.,* 2005, vol. 18, 585-596vii **[0009]**
- **SCHWARTZ et al.** *J Glaucoma,* 1996, vol. 5, 427-432 **[0009]**
- **WALKER ; ROSEN.** *Age Ageing,* 2006, vol. 35, 332-335 **[0010]**
- **MUDHER ; LOVESTONE.** *Trends Neurosci.,* 2002, vol. 25, 22-26 **[0010]**
- **LYKETSOS et al.** *Am J Geriatr Psychiatry,* 2006, vol. 14, 561-572 **[0011]**
- **DEKOSKY et al.** *Contemp Clin Trials,* 2006, vol. 27, 238-253 **[0011]**
- **AREOSA-SASTRE et al.** *Cochrane Database Syst Rev.,* 2004, vol. 18, CD003154 **[0011]**
- **FLDER et al.** *Mt Sinai J Med.,* 2006, vol. 73, 931-940 **[0011]**
- **BRINTON ; WANG.** *Curr Alzheimer Res.,* 2006, vol. 3, 185-190 **[0011]**
- **KELLEHER-ANDERSSON.** *Curr Alzheimer Res.,* 2006, vol. 3, 55-62 **[0011]**
- **GREENBERG ; JIN.** *Curr Alzheimer Res.,* 2006, vol. 3, 25-8 **[0011]**
- **HARDY ; GWINN-HARDY.** *Science,* 1998, vol. 282, 1075-1079 **[0012]**
- **MARTIN.** *N Engl J Med,* 1999, vol. 340, 1970-1980 **[0012]**
- **SCHOLS et al.** *Ann Neurol.,* 1997, vol. 42, 924-932 **[0012]**
- **BONELLI ; HOFMANN.** *Expert Opin Pharmacother,* 2004, vol. 5, 767-776 **[0014]**
- **KARPUJ et al.** *Nat Med.,* 2002, vol. 8, 143 149 **[0014]**
- **BROOKS et al.** *Proc Natl Acad Sci USA,* 2002, vol. 99, 6216-6221 **[0015]**
- **COMPSTON ; COLES.** *Lancet,* 2002, vol. 359, 1221-1231 **[0017]**
- **BRUCK ; STADELMANN.** *Neurol Sci.,* 2003, vol. 24, S26S-S267 **[0018]**
- **HOHLFELD.** *Int MS J,* 2003, vol. 10, 103-105 **[0018]**
- **YANG et al.** *J Neurosci.,* 2006, vol. 26, 2157-2166 **[0019]**
- **OKANO.** *Ernst Schering Res Found Workshop,* 2006, vol. 60, 215-228 **[0019]**
- **SHORS et al.** *Nature,* 2001, vol. 410, 372-376 **[0020]**
- **ALLAIN et al.** *Psychol Neuropsychiatr Vieil,* 2003, vol. 1, 151-156 **[0020]**

- **THOMPSON et al.** *Proc Natl Acad Sci USA,* 2001, vol. 98, 11650-11655 **[0021]**
- **CANNON et al.** *Proc Nat] Acad Sci USA,* 2002, vol. 99, 3228-3233 **[0021]**
- **CAZENEUVE ; CAILLOT.** *Jahresb. der Pharm.,* 68 **[0040]**
- **STEVENSON.** *Amer. Jour. Pharm.,* 513 **[0040]**
- **DHULEY et al.** *Indian J Exp Biol.,* 1993, vol. 31, 443-445 **[0045]**
- **MUJUMDAR et al.** *Jpn J Med Sci Biol.,* 1990, vol. 433, 95-100 **[0045]**
- **KHAJURIA et al.** *Mol Cell Biochem.,* 1998, vol. 189, 113-118 **[0046]**
- **ATAL et al.** *J Pharmacol Exp Ther.,* 1985, vol. 232, 258-262 **[0047]**
- **BADMAEV et al.** *Nutr Res.,* 1999, vol. 19, 381-388 **[0047]**
- **BADMAEV et al.** *J Nutr Biochem.,* 2000, vol. 11, 109-113 **[0047]**
- **BANO et al.** *Eur J Clin Pharmacol.,* 1991, vol. 41, 615-617 **[0047]**
- **PATTANAIK et al.** *Phytother Res.,* 2006, vol. 20, 683-686 **[0047] [0087]**
- **PEI.** *Epilepsia,* 1983, vol. 24, 177-182 **[0051] [0056]**
- **LIU et al.** *Biochemical Pharmacology,* 1984, vol. 33, 3883-3886 **[0051] [0055] [0056]**
- **D'HOOGE et al.** *Arzneimitteltorschung,* 1996, vol. 46, 557-560 **[0052]**
- **LIU ; SIMON et al.** *J Neurophysiol.,* 1996, vol. 76, 1858-1869 **[0054] [0057]**
- **MCNAMARA et al.** *Br J Pharmacology,* 2005, vol. 144, 781-790 **[0054]**
- **VARGA et al.** *Neurosci Lett.,* 2005, vol. 385, 137-142 **[0054]**
- **GUNTHROPE et al.** *Neuropharmacology,* 2004, vol. 46, 133-146 **[0054]**
- **PEL.** *Epilepsia,* 1983, vol. 24, 177-182 **[0055]**
- **JIN et al.** *Proc. Natl. Acad. Sci. USA,* 2001, vol. 98, 4710-4715 **[0060]**
- **JIANG et al.** *Stroke,* 2001, vol. 32, 1201-1207 **[0060]**
- **KEE et al.** *Exp. Brain. Res.,* 2001, vol. 136, 313-320 **[0060]**
- **PERFILIEVA et al.** *J- Cereb. Blood Flow Metab.,* 2001, vol. 21, 211-217 **[0060]**
- **ERIKSSON et al.** *Nat Med.,* 1998, vol. 4, 1313-1317 **[0060]**
- **VAN PRAAG et al.** *Nature,* 2002, vol. 415, 1030-1034 **[0060] [0061]**
- **GAGE et al.** *J Neurobiol,* 1998, vol. 36, 249-266 **[0060]**
- **SCHNEIDER et al.** *J Clin Investigation,* 2005, vol. 115, 2083-2098 **[0068]**
- **GUAN et al.** *Cell Tissue Res.,* 2001, vol. 305, 171-176 **[0068]**
- **JACOBS et al.** *Proc. Natl. Acad. Sci. USA,* 2006, vol. 103, 3902-3907 **[0068]**
- **ALMER et al.** *J Neurochem,* 1999, vol. 72, 2415-2425 **[0074]**
- **JANUS ; WESTAWAY.** *Physiol Behav,* 2001, vol. 73, 873-886 **[0074]**
- **SATHASIVAM et al.** *Philos Trans R Soc Lond B Biol Sci.,* 1999, vol. 354, 963-969 **[0074]**
- **AL-MAHDAWI.** *Genomics,* 2006, vol. 88, 580-590 **[0074]**
- **LUDOLPH.** *J Neurol,* 2000, vol. 247, VI/13-VI/18 **[0079]**
- **LYRITIS ; TROVAS.** *Bone,* 2002, vol. 30, 71S-74S **[0084]**
- **DHILLO ; BLOOM.** *Curr Opin Pharmacol,* 2001, vol. 1, 651-655 **[0084]**
- **THORNE ; FREY.** *Clin Pharmacokinet,* 2001, vol. 40, 907-946 **[0084]**
- **TIRUCHERAL et al.** *Expert Opin Biol Ther,* 2001, vol. 1, 49-66 **[0084]**
- **JIN et al.** *Ann Neurol,* 2003, vol. 53, 405-409 **[0084]**
- **LEMERE et al.** *Neurobiol Aging,* 2002, vol. 23, 991-1000 **[0084]**
- **LAWRENCE.** *Lancet,* 2002, vol. 359, 1674 **[0084]**
- **LIU et al.** *Neurosci Lett,* 2001, vol. 308, 91-94 **[0084]**
- Remington's Pharmaceutical Sciences. Mack Publishing Co, 1990 **[0085]**
- **SUNKARA.** *Pharmazie,* 2001, vol. 56, 640-642 **[0087]**
- **BOXENBAUM ; DILEA.** *J Clin Pharmacol,* 1995, vol. 35, 957-966 **[0087]**
- **EDITH MATHIOWITZ.** Encyclopedia of Controlled Drug Delivery. John Wiley & Sons, 1999, vol. 2, 896-920 **[0089]**
- **EMERICH et al.** *Clin Pharmacokinet,* 2001, vol. 40, 105-123 **[0090]**
- **SIEGAL et al.** *Clin Pharmacokinet,* 2002, vol. 41, 171-186 **[0090]**
- **MAHMOOD et al.** *Neurosurgery,* 2001, vol. 49, 1196-1204 **[0097]**
- **LU et al.** *J Neurotrauma,* 2001, vol. 18, 813-819 **[0097]**
- **LU et al.** *Cell Transplant,* 2002, vol. 11, 275-281 **[0097]**
- **LI et al.** *Neurology,* 2002, vol. 59, 514-523 **[0097]**
- **MAURER et al.** *Proteome Sci.,* 2003, vol. 1, 4 **[0101]**
- **DENNIS et al.** *Gene,* 2002, vol. 294, 269-277 **[0102]**